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Study of the role of the human TREX-2 complex in the DNA Damage Response

Etude du rôle du complexe humain TREX-2 lors de la Réponse aux Dommages de l'ADN

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List of Abbreviations

53BP1	p53 Binding Protein 1
AID	Activation Induced citidine Deaminase
ALT-EJ	Alternative End Joining
ATM	Ataxia Telangiectasia Mutated
ATR	Ataxia Telangiectasia and Rad6 Related
ATXN7	Ataxin 7
ATXN7L3	Ataxin 7 Like 3
BARD1	BRCA1 Associated Ring Domain 1
BIR	Break Induced Replication
BLM	Bloom
BRCA1	Breast Cancer 1
BRCA2	Breast Cancer 2
BRCT	Brca1 C-Terminus
CDK	Cycline Dependent Kinase
ChIP	Chromatin Immunoprecipitation
CID	Cdc31-Interacting Domain
CO	Crossover
CSR	Class Switch Recombination
CtIP	CtBP Interacting Protein
DDR	DNA Damage Response
dHJ	Double Holliday Junction
DNA-Pk	DNA-dependent Protein Kinase
DNA-PKcs	DNA Protein Kinase catalytic subunit
DSB	Double Strand Break
dsDNA	Double Stranded DNA
DUB	Deubiquitinase
ENY2	Enhancer of Yellow 2
EXO1	Exonuclease 1
FHA	Fork Head Associated
FRAP	Fluorescence Recovery After Photobleaching
GANP	Germinal center Associated Nuclear Protein
GC	Germinal Center

GCN5	General Control of aminoacids synthesis protein 5
HAT	Histone Acetyltransferase
HJ	Holliday Junction
HR	Homologous Recombination
Ig	Immunoglobulin
IR	Ionizing Radiation
IRIF	Irradiation Induced Foci
KD	Knock-Down
KO	Knock-Out
LADs	Lamin Associated Domain
MCD1	Mediator of DNA Damage Checkpoint protein 1
MCM3AP	Minichromosome Maintenance 3 Associated Protein
MEFs	Mouse Embryonic Fibroblasts
MNase	Micrococcal Nuclease
MRE11	Meiotic Recombination 11 homolog 1
MRN	MRE11-RAD51-NBS1
MRX	Mre11-Rad50-Xrs2
MS	Mass Spectrometry
NBS1	Nijmegen Breakage Syndrome 1
NCO	Non Crossover
NE	Nuclear Envelope
NHEJ	Non-Homologous End Joining
NPC	Nuclear Pore Complex
PALB2	Partner And Localizer of BRCA2
PARP	Poly ADP-rybose Polymerase
PTMs	Post Translational Modifications
RAP80	Receptor Associated Protein 80
RING	Really Interesting New Gene
RNF20	Ring Finger protein 20
RNF40	Ring Finger protein 40
RPA	Replication Protein A
SAGA	Spt-Ada-Gcn5 Acetyltransferase
ssDNA	Single Stranded DNA
TIP60	Tat Interactive Protein 60

TPR	Translocated Promoter Region
TREX-2	TRanscription and EXport complex 2
UDR	Ubiquitin Dependent Recruitment motif
UIM	Ubiquitin Interacting Motif
USP	Ubiquitin Specific Peptidase
WT	Wild-Type
XLF	XRCC4-Like Factor
XRCC	X-Ray repair Cross Complementing

Thesis summary in French

L'intégrité de l'information génétique est essentielle pour assurer le bon fonctionnement de la cellules afin d'éviter l'instabilité génomique, une des caractéristiques principales du cancer. Il a été estimé que l'ADN subit environ à 70 000 lésions par jour dont 75% sont des lésions simple brin (SSBs) pouvant potentiellement être converties en cassures double brin (DSB : Double Strand Breaks). Bien que les DSBs soient moins fréquentes, elles sont plus dangereuses pour la cellule si elles ne sont pas réparées pouvant ainsi conduire à l'instabilité génomique. En effet, les DSBS peuvent entrainer des translocations impliquées dans le processus de carcinogenèse. Au sein de chaque cellule, en fonction du type de lésion, plusieurs mécanismes permettent de réparer l'ADN. Suite à des DSBs, la voie de signalisation de Réponse aux Dommages de l'ADN (DDR: DNA Damage Response) est activée. Cette voie de signalisation est caractérisée par le recrutement des protéines effectrices au site de lésion. Bien que la voie DDR intervienne immédiatement après la survenue d'une cassure double brin, la réparation est médiée par plusieurs voies sous-jacentes qui dépendent du cycle cellulaire et font interevenir différentes protéines effectrices. Les deux voies de signalisation sous-jacentes principales sont la Jonction d'extrémités non homologues (NHEJ: Non Homologous End Joining) et la Recombinaison Homologue (HR: Homologous Recombination). La voie NHEJ répare la cassure en liant les deux extrémités de deux brins mais avec la persistance d'insertions ou de délétions au site de lésion, créant-ainsi des erreurs dans l'ADN après réparation. La voie NHEJ est la voie privilégiée en phase G1 du cycle cellulaire, bien qu'elle soit aussi active pendant toutes les phases du cycle cellulaire. Au contraire, la voie HR ne cause pas d'erreurs étant donné que la réparation utilise le chromosome homologue, non endommagé, comme modèle. Pour cette raison, c'est la voie privilégiée en phase S et G2 du cycle cellulaire. L'étape cruciale au cours de la recombinaison homologue est la résection de l'extrémité de l'ADN : lorsque l'extrémité 5' simple brin est digérée pour laisser une longue extrémité 3' simple brin qui va envahir l'ADN double brin homologue et ainsi servir de modèle pour la réparation. La résection se déroule pendant les phases S et G2, lorsque les chromosomes homologues sont accessibles. Ce processus inhibe la voie NHEJ au profit de la voie HR. Le succès de la réparation dépend de l'efficacité des facteurs impliqués et de l'efficacité de la signalisation, qui dépend des modifications post-traductionnelles (PTMs) des histones et des protéines non-histones. La signalisation débute par une cascade de phosphorylations qui induisent la phosphorylation du variant d'histone H2AX

(γH2AX) sur la sérine 139 et correspond à un des premiers événements. La très forte compaction de l'ADN sous forme de chromatine à l'intérieur du noyau cellulaire, peut être modulée par des modifications d'histones qui ont donc un rôle très important dans la réparation de l'ADN. Des études récentes ont montrées que l'efficacité de réparation dépend de PTMs spécifiques ainsi que de la localisation dans l'espace nucléaire de l'ADN endommagé. Plusieurs études chez la levure ont montrées que, pour être réparée plus efficacement, les cassures DSBs peuvent être relocalisées à d'autres endroits que là où le dommage a eu lieu initialement pour une meilleure efficacité. Un de ces sites préférentiels pour la réparation est le complexe du pore nucléaire (NPC), qui est un canal connectant le noyau et le cytoplasme, dont la fonction principale est la communication entre les deux compartiments. Pour cette raison, il apparait surprenant que cette structure puisse être également impliquée dans la réparation de l'ADN. Plus récemment, il a aussi été montré dans les cellules de mammifères, que les protéines du pore nucléaire (les nucléoporines) ont un rôle dans la réponse DDR.

De plus, plusieurs protéines adaptatrices peuvent servir d'intermédiaire pour l'interaction entre le NPC et le noyau, et ainsi conférer au NPC un rôle dans la maintenance de la stabilité génomique. Un exemple de protéine adaptatrice chez la levure est le complexe coactivateur Spt-Ada-Gcn5-Acetyltransferase (SAGA) ainsi que le complexe TRanscription and EXport 2 (TREX-2). Chez la levure l'interaction physique et fonctionnelle entre les deux complexes connecte la transcription en cours avec le pore nucléaire pour l'export immédiat.

Dans ce contexte, la connexion entre la transcription et l'export chez la levure a été démontrée comme étant requise pour la maintenance de l'intégrité du génome. Les mutants TREX-2 présentent un phénotype d'hyper-recombinaison, une des caractéristiques principales de l'instabilité génomique dans les cellules de levure. En revanche, la situation est très différente dans les cellules de mammifères dans lesquelles la transcription et l'export sont deux processus distincts. Le rôle des nucléoporines ainsi que leurs protéines associées dans la DDR ne semble pas lié à l'export des ARN ou à la transcription.

Dans ce contexte, le but de mon projet est de déterminer la relation entre le complexe TREX-2 associé au complexe du pore nucléaire et la stabilité du génome dans les cellules humaines.

Le complexe TREX-2, est composé de cinq sous-unités présentant une forte homologie de séquence et de structure de la levure aux mammifères. TREX-2 est formé par

l'association de la protéine architecturale GANP, de deux sous-unités ENY2, et d'une copie des protéines PCID2, DSS1 et CENTRIN (Sac3, Sus1, Thp1, Sem1 et Cdc31 chez la levure). ENY2/Sus1 est présente en double dans le complexe TREX-2 et est également présente dans le module de Deubiquitination (Nagai et al.) du complexe SAGA, dont la fonction est de retirer l'ubiquitine de l'histone H2B. Contrairement à la levure, les expériences d'immunopréicipitation (IP) couplées à l'analyse par spectrométrie de masse (MS) dans les cellules de mammifère ont montré que SAGA et TREX-2 sont deux complexes distincts, ne partageant que la sous-unité ENY2. Aucune autre sous-unité de TREX-2 n'est associée avec SAGA.

Afin de déterminer le rôle potentiel de TREX-2 dans la stabilité génomique, nous avons décidé d'analyser la réponse cellulaire face à un dommage de l'ADN causé par des drogues en absence de certaines sous-unités spécifiques de TREX-2. Parmi les cinq sous-unités du complexe TREX-2, la protéine architecturale GANP est la plus représentative de la fonction et de l'intégrité du complexe. De fait, une déplétion de GANP dans les cellules humaines a pour conséquences une perte de la localisation de TREX-2 au NPC et l'accumulation d'ARNm dans le noyau, démontrant ainsi que la fonction d'export des ARNm est également affectée. La déplétion d'ENY2 cause le même phénotype mais étant donné son association avec SAGA, nous avons décidé de déterminer la fonction de TREX-2 par une déplétion de GANP.

Nos résultats montrent que les cellules avec un faible niveau de GANP résiduel sont plus sensibles, que des cellules contrôles, aux DSBs induites par la phléomycine qui est une drogue radiomimétique. Cette observation est accompagnée d'un défaut de la DDR qui est démontré par un retard de la réparation des cassures et une phosphorylation persistante des kinases de signalisation sans affecter l'action précoce de la DDR. Etant donné la localisation de GANP au niveau du NPC, nous avons cherché à déterminer si les cassures non réparées s'accumulent en périphérie du noyau. Cependant, les analyses de la distribution de l'intensité de γH2AX n'ont pas montré d'accumulation des cassures non réparées en périphérie, suggérant un effet plus global.

Pour rappel, une seule cascade de signalisation caractérise la voie DDR après la détection d'une cassure, se séparant ensuite en deux voies sous-jacentes principales : NHEJ et HR. Nos résultats ont montré que le défaut de réparation lors de la déplétion de GANP n'est pas médié par une altération des étapes initiales de la DDR, suggérant que GANP est impliqué dans les étapes plus tardives. L'utilisation des lignées cellulaires U2OS contenant une cassette reporter DR-GFP, permettant de mesurer l'efficacité des

voies NHEJ et HR suite à une cassure induite par IsceI dans la cassette DR-GFP, nous a permis d'identifier un rôle spécifique de GANP dans la voie HR, dont les premières étapes de résection sont affectées.

La déplétion de GANP s'accompagne effectivement d'une diminution importante du recrutement de tous les facteurs de résection.

Afin d'étudier le rôle du complexe TREX-2 dans la DDR, nous avons décidé de tester si la déplétion d'autres sous-unités du complexe a le même effet sur la DDR. Nous avons ainsi testé la fonction d'ENY2, bien qu'ENY2 soit présente dans au moins un autre complexe. De manière surprenante, la déplétion d'ENY2 a l'effet opposé, c'est à dire une forte augmentation de l'efficacité de HR.

ENY2 fonctionne comme une protéine adaptatrice dans le module DUB de SAGA, composé d'ENY2, d'ATXN7, d'ATXN7L3 et de l'enzyme USP22 qui catalyse le retrait de l'ubiquitine de la Lysine (K) 120 de l'histone H2B mono-ubiquitinée (H2Bub1). La marque H2Bub1 est associée avec l'élongation de la transcription et il a été montré récemment que la monoubiquitination de H2B intervient en réponse à un dommage induit dans l'ADN après une irradiation. Bien que le recrutement de RNF20/40, qui catalyse l'ubiquitination d'H2B, après un dommage dans l'ADN a déjà été étudié, peu de choses concernant la régulation de la déubiquitination et de l'importance de l'équilibre entre H2B et H2Bub1 en réponse à un dommage de l'ADN sont connues

Grâce à l'analyse des niveaux de H2Bub1 lors de la déplétion de GANP ou ENY2, avec ou sans induction de dommage de l'ADN, nous avons montré que contre toute attente que la dynamique de H2Bub1 ne dépend pas seulement de la présence d'ENY2 dans le module DUB du complexe SAGA, mais aussi de la stabilité de TREX-2 pour maintenir un bon équilibre entre ubiquitination et déubiquitination. Comme attendu, les cellules dépourvues d'ENY2 ont des niveaux d'H2Bub1 plus élevés mais de manière surprenante les cellules sans GANP ont des niveaux de H2Bub1 réduits, avant et après dommage de l'ADN.

Après une double déplétion de GANP et ENY2, les niveaux de H2Bub1 sont similaires à ceux de cellules contrôles (avec ou sans induction de dommages de l'ADN) suggérant que l'interaction d'ENY2 avec le complexe TREX-2 a un rôle fondamentale dans l'équilibre entre ubiquitination et déubiquitination de H2B et que cet équilibre est essentiel à une réparation efficace pour la voie HR. De plus, nous montrons que la déplétion d'ENY2 à partir de cellules ayant des niveaux réduits de GANP permet de restaurer complètement le recrutement de la protéine RAD51 en charge de la résection au niveau du site IsceI

suggérant un lien entre l'équilibre de H2Bub1 pour la HR et que cette redistribution dépend de l'intégrité du complexe TREX-2.

En conclusion, notre étude a montré que le complexe TREX-2 humain est fondamental pour la réponse face à un dommage de l'ADN et pour une réparation efficace et que ce processus nécessite le couplage entre TREX-2 et SAGA pour la régulation des niveaux d'H2Bub1.

Thesis Summary

The maintenance of proper genetic information is essential to ensure correct cellular functions and to avoid genomic instability that is a hallmark of cancer. It has been approximated that the DNA is challenged by 70.000 lesions per day of which 75% correspond to single stranded DNA breaks (SSBs) that could be eventually converted into double stranded DNA breaks (DSB), in which both strands are broken. DSBs are less common but more dangerous for the cell because, if unrepaired, they can lead to genomic instability, translocations and eventually cancer. Cells have evolved different pathways to repair their genome, accordingly to the type of lesion. In response to DSBs they initiate a signalling cascade, named the DNA Damage Response (DDR). This signalling pathway is characterized by the recruitment and the extensive spreading of DDR proteins around the lesions. Although the DDR response involves one main common pathway which intervenes immediately after the insurgence of the break, the repair is accomplished following different sub-pathways, depending on the cell cycle stage, and is mediated by different proteins. Two main sub-pathways exist: Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR). The former ligates the extremities of the two ends and often leaves insertions or deletions at the site of break, thus being error-prone. Although NHEJ is active throughout the cell cycle, it is the favourite pathway during the G1 phase. HR is instead by definition error-free, because the break is repaired using as a template the homologous chromosome; for this reason it is the preferred pathway during the S and G2 phases of the cell cycle. The decisive step in HR is DNA end resection, in which the 5' ssDNA end of the blunt end is digested to leave a long 3' end ssDNA filament that will invade the homologous double strand of DNA to use it as a template for repair. The resection process happens in S and G2 phases, when there is availability of homologous chromosomes, inhibiting NHEJ and favouring HR.

The successful completion of the repair is driven by the efficiency of all the factors involved and by the efficient signalling, that relies on different post-translation modifications (PTMs) starting from a phosphorylation cascade that, as one of the earliest events, induces the phosphorylation of the histone variant H2AX on serine 139 (γH2AX). It is not surprising that histones modifications play a key role in DNA repair if we consider that the repair has to be completed in the context of chromatin, but also in the highly compartmentalized environment of the nucleus. Recent studies have shown that the efficiency of the repair and the choice of the pathway are made also accordingly to the chromatin environment, characterized by specific histone PTMs and to the spatial

localization of the damaged DNA. Several studies in budding yeast (y) have shown that DSBs can relocate to different sites from where they have been generated for efficient repair. One of these sites, in yeast, is the Nuclear Pore Complex (NPC), the channel that connects the nucleus with the cytoplasm, whose main function is indeed the communication and transport between the two compartments. For this reason it appeared surprising that these structures had a role in DNA repair. More recently it has been shown, also in human (h) cells, that proteins of the nuclear pore (so-called nucleoporins) have a role in the DDR.

In addition many adaptor proteins might mediate the interaction between the NPC and the inner nuclear environment and contribute to the additional roles of the nuclear pore in genome stability maintenance. Important examples of these adaptors in yeast are the Spt-Ada-Gcn5-Acetyltransferase (SAGA) co-activator complex and the TRanscription and EXport complex 2 (TREX-2). In yeast the physical and functional interaction between the two complexes connects the ongoing transcription with the nuclear pore for immediate export. This connection has been shown to be required for the maintenance of genome integrity, and mutants of the TREX-2 complex show hyper-recombination phenotype, a hallmark of genomic instability in yeast cells. In mammalian cells, the situation is different as transcription and export are two distinct processes and the involvement of nucleoporins and associated proteins in the DDR has not been connected with RNA export or transcription defects.

In this context the aim of my project was to investigate the relationship between the NPC-associated TREX-2 complex and genomic stability in human cells.

The TREX-2 complex is composed of five subunits both in yeast and mammals whose crystal structure reveals a strong homology. hTREX-2 is composed by the protein GANP, two ENY2 subunits, PCID2, DSS1 and CENTRIN (respectively Sac3, Sus1, Thp1, Sem1 and Cdc31 in budding yeast). Interestingly, the hENY2/ySus1 subunit is present twice in the complex and is also found associated with the Deubiquitination (DUB) module of the SAGA complex, whose function is to remove ubiquitin from Lysine (K) 20 (K23 in yeast) of monoubiquitinated histone H2B (H2Bub1). Differently from yeast, immunoprecipitation (IP) experiment followed by Mass Spectrometry (MS) in mammalian cells showed that SAGA and TREX-2 are two distinct complexes and while they both co-immunoprecipitate with ENY2, no other TREX-2 subunit is found associated with SAGA.

To investigate the possible role of the TREX-2 complex in genome stability in human cells we decided to analyse the cell response to DNA damaging drugs in the absence of TREX-

2 specific subunits. Among the five subunits that form the TREX-2 complex the scaffold protein GANP is the main representative of the function and responsible for the integrity of the complex. In fact GANP knock-down (KD) in human cells results in impairment of TREX-2 localization at the NPC and accumulation of mRNA in the nucleus. ENY2 KD results in the same defects, but given its association with SAGA we decided to start to investigate TREX-2 function in GANP KD.

Our results show that cells depleted of GANP are more sensitive, compared to controls, to the DSBs-inducing radiomimetic drug phleomycin. This is accompanied by a defect in the DDR demonstrated by delayed repair of breaks and a persistent phosphorylation of signalling kinases without affecting the early activation of the DDR. Given the NPC localization of GANP we investigated whether the unrepaired breaks were accumulated at the periphery of the nucleus; however analyses of γH2AX intensity distribution showed no specific accumulation of breaks at the periphery suggesting a more global effect.

As mentioned before, a unique signalling cascade characterizes the DDR pathway after break recognition that diverges in two main pathways of repair, NHEJ and HR. Our results showed that repair defects in GANP KD are not mediated by alterations in the initial steps of the DDR suggesting the involvement of GANP at later steps. With the utilization of two specific U2OS cell lines, containing a DR-GFP cassette allowing the measurement of the HR or NHEJ efficiency after a unique DSB induction, we showed a specific role of GANP in the HR pathway, which is defective from the early stages of resection in the absence of GANP. Indeed, recruitment of all tested resection factors at a single DSB was severely impaired in GANP KD cells.

To further investigate the role of the whole complex in the DDR we decided to test whether depletion of another subunit of the TREX-2 complex would have the same effect on the DDR. Surprisingly depletion of ENY2 had the opposite effect, showing a strong increase in HR efficiency.

ENY2 functions as an adaptor protein in the DUB module of SAGA, composed of ENY2, ATXN7, ATXN7L3 and the enzyme USP22 that catalyses the removal of monoubiquitin from the H2Bub1.

We ascribed the increase in HR efficiency in ENY2-depleted cells to the disruption of the SAGA DUB because depletion of a second subunit of the DUB, ATXN7L3, showed the same increase.

H2Bub1 mark is associated with transcription elongation and it has recently been shown that monoubiquitination of H2B occurs in response to irradiation-induced DNA damage.

While the recruitment of RNF20/40 catalysing the formation of H2Bub1 after DNA damage has been extensively studied, little is known about the regulation of its deubiquitination and the importance in the balance between H2B and H2Bub1 in the response to DNA damage. Analysing the levels of H2Bub1 in GANP and ENY2 KD cells, with or without damage induction, we show that, unexpectedly, the dynamic of H2Bub1 mark is not only dependent on ENY2 presence, as a result of the DUB stability, but the stability of the TREX-2 complex is also an important factor for the correct balance between ubiquitination and deubiquitination. As expected cells lacking ENY2 showed higher basal levels of H2Bub1 but surprisingly cells depleted of GANP had lower amount of global H2Bub1, before and after damage.

The double KD of GANP and DUB subunits ENY2 or ATXN7L3 restored H2Bub1 to control levels, (both in undamaged and damaged cells) suggesting that the interaction of ENY2 with the TREX-2 complex plays a fundamental role in balancing the DUB activity. Moreover we ascribed the repair defects of GANP and ENY2-depleted cells to the disrupted H2B/H2Bub1 balance because the additional DUB depletion on cells already depleted of GANP completely rescues the recruitment of resection protein RAD51 at the unique DSB site.

In summary our study showed that the human TREX-2 complex, is fundamental for efficient DNA damage repair in human cells and that this process involves interplay between TREX-2 and SAGA in regulating H2Bub1 levels.

1. INTRODUCTION

1.1. DNA damage: a threat for genome integrity

The genome is a bag of information encoded by the DNA that has to be passed as faithfully as possible to the next generation. The maintenance of its integrity is fundamental to the life of the single cell and of the organism. However the DNA is constantly challenged by endogenous and exogenous damaging agents that pose a threat to its stability. For this reason the cell has evolved a series of protection mechanisms to copy with the huge amount of damage that the DNA receive every day, estimated to be around 10^3 to 10^6 molecular lesions (Lindahl, 1993; Lindahl and Barnes, 2000).

Endogenous damage occurs generally at higher rate compared to exogenous damage and among the several causes of endogenous damage the majority are represented by the incorporation of ribonucleotides during DNA synthesis, and by Reactive Oxigen Species (ROS) produced by oxidation processes in the cell. ROS can react with the DNA molecule inducing bases modifications that lead to mismatches in the double helix of DNA. Physiological processes such as replication or collisions between the replication fork and the transcription machinery can pose a threat to the genome. Stalling of the replication fork, process know as replication stress, can lead to Double Strand Breaks (DSBs) (Helmrich et al., 2013; Techer et al., 2017). Moreover DNA damages such as DSBs or deamination of cytidines can happen in a programmed and regulated manner in biological process as meiosis or in the immune response (Alt et al., 2013; Baudat et al., 2013).

Exogenous sources of DNA damage are mainly represented by Ultraviolet (UV) light, which generates bases modifications that create DNA helix distorstion (Karran and Brem, 2016; Park and Kang, 2016). Ionizing radiation (IR) used in radiotherapy and chemotherapic agents can create different kind of damages such as DSBs, Single Stranded Breaks (SSBs) and base lesions (Mavragani et al., 2017).

Given the complexity of DNA lesions that can occur in the genome cells have evolved different kind of pathways to repair them faithfully. For example base modifications created by UV, IR and chemotherapy or breaks arising in one strand of the double helix, can be repaired by the Nucleotide Excision Repair (NER), Base Excision Repair (BER) and the Mismatch-Mediated Repair (MMR) pathway (Liu et al., 2017; Scharer, 2013; Wallace, 2014).

DSBs are much less frequent than base modifications but at the same time they are among the most dangerous type of lesions because if unrepaired they can lead to unwanted chromosome rearrangements such as translocations and genome instability.

1.2 The DNA Damage Response (DDR); from break recognition to signal spreading

DNA DSBs activate a complex signalling cascade called the DNA Damage Response (DDR) that relies on a myriad of post-translational modifications (PTMs) that alter the specificity of protein-protein interaction and their catalytic activity. Recruitment of a plethora of proteins at the damage sites is controlled by PTMs and ensures that the damage is faithfully repaired using alternative pathways (reviewed in (Thompson, 2012)). Several DDR factors are involved in the recognition of the damage that will be covered by subsequent recruitment of proteins forming the so-called Irradiation-Induced-Foci (IRIF) (Bekker-Jensen and Mailand, 2010). Break repair happens on the basis of different non-redundant pathways, which rely on unique proteins and signalling mechanisms; however the first steps of the DDR are recognized as a single common pathway relying on activation of apical kinases that will recognize the breaks and start a signalling cascade (Figure 1).

γH2AX

One of the first events that characterizes the DDR is the phosphorylation of the histone variant H2AX on serine 139 γH2AX), that was characterized as an immediate response to irradiation-induced damaged and other DNA damaging agents (Takahashi and Ohnishi, 2005) (reviewed in (Kinner et al., 2008; Lobrich et al., 2010; Stucki and Jackson, 2006)). Phosphorylation of H2AX is performed in a redundant way by master regulators of the DDR, apical kinases that control PTMs of a variety of downstream effectors, Ataxia Telangiectasia Mutated (ATM), DNA dependent Protein Kinase (DNA-PK) and Ataxia Telangiectasia and Rad3 related (ATR) (An et al., 2010; Burma et al., 2001; Lou et al., 2006; Stiff et al., 2004). While ATM and DNA-PK respond principally to DSBs, ATR is activated in response to a variety of damages, being recruited via ssDNA coated by the ssDNA binding protein Replication Protein A (RPA) (Zou and Elledge, 2003). The principal kinase responsible for H2AX phosphorylation is generally considered ATM because it has been shown that Mouse Embryonic Fibroblasts (MEFs) depleted for DNA-PK still support γH2AX formation as Wild-Type (WT) MEFs while ATM-depleted mouse and human fibroblasts show delayed phosphorylation (Stiff et al., 2004). Interestingly ATM-depleted human lymphoblasts fail completely to form γH2AX. γH2AX formation at the sites of breaks plays a critical role in for the recruitment of downstream effectors (Celeste et al., 2002; Lou et al., 2006) that forms platforms for the recruitment of subsequent proteins in a cascade that has as main goal the repair of the DSB. Loss of H2AX is compatible with life and H2AX null mice are viable with reduced fertility (Celeste et al., 2002) but consistently with

its role in repair H2AX null mice MEFs and Embryonic Stem (ES) cells are 2- to 3-fold more sensitive to irradiation than control cells and shown elevated chromosomal instability even without irradiation (Bassing et al., 2002; Celeste et al., 2002). H2AX is also phosphorylated on tyrosine 142 by the chromatin remodeler Williams Beuren Syndrome Transcription Factor (WSTF) (Xiao et al., 2009); however the role of this modification in DNA repair is not clarified yet. It has been proposed that this phosphorylation might interfere with the more canonical phosphorylation on ser139 and that it is needed to restrain γH2AX at the vicinity of the break (Stucki, 2009).

Ataxia Telangiectasia Mutated (ATM)

ATM is thus a key player in the DDR (reviewed in (Derheimer and Kastan, 2010; Lavin, 2008; Lavin and Kozlov, 2007; Lee and Paull, 2007)) that induces γH2AX formation and spreading helping the formation of a platform for proteins docking. ATM is usually activated in response to damage, and this activation is generally attributed to its interaction with the MRE11-RAD51-NBS1 (MRN complex; described hereafter) after break recognition (reviewed in(Lavin, 2007; McKinnon, 2012). However activated ATM is detectable at very low levels of damage (≈3 DSBs per cell) (Bakkenist and Kastan, 2003) thus is possible that ATM might be activated at long distance from the break and then recruited to the site of break through MRN interaction (Bakkenist and Kastan, 2003). One simple model implies chromatin modifications at the basis of initial ATM activation (Bakkenist and Kastan, 2003) where chromatin relaxation plays a key role. Indeed forced chromatin relaxation can activate ATM even in the absence of detectable DSBs (Rogakou et al., 1999; Rogakou et al., 1998; Stucki and Jackson, 2006). Only after ATM activation through chromatin modification the MRN complex would play a role in recruiting activated ATM at the vicinity of the break (Bakkenist and Kastan, 2003). In any case regulation of ATM activity seems to be a job shared by different regulators. First among all, ATM itself autophosphorylates on three different phosphorylation sites. Serine 1981 is probably the most known phosphorylated site (Goodarzi et al., 2004), but serine 1983 and serine 367 have also been shown to play a role in ATM activation (Kozlov et al., 2006). Indeed the three phosphorylation-defective mutants are all defective in phosphorylation of ATM substrates (Kozlov et al., 2006) but ser1983 and ser367 seems not be strictly fundamental for γH2AX formation as instead ser1981 (Kozlov et al., 2006). However ser1981 phosphorylation alone is not sufficient to activate ATM, which remains as an inactive dimer. Interestingly activation of ATM relies in large part on acetylation on Lysine (K) 3016 by the Histone Acetyltransferase (HAT) Tip60 (Sun et al., 2005). A constitutive complex

Tip60-ATM was shown to be important in the early activation of ATM upon DSBs (Sun et al., 2005). Tip60 acetylates ATM in response to irradiation concomitantly with ATM autophosphorylation (Sun et al., 2005); consistent with these data Tip60 localizes at the sites of breaks in foci co-localizing with ATM^{s1981p} and γH2AX (Sun et al., 2005). The acetylation event seems to be sufficient to induce monomerization and thus activation of ATM (Sun et al., 2007). Recruitment of Tip60 at DSBs seems to rely on MRN complex (Sun et al., 2005) and on the presence of the trimethylated version of K9 on Histone H3 (H3K9me3) (Sun et al., 2009). ATM also phosphorylates p53 and Chk2 to block the cell cycle in G2/M phase (Matsuoka et al., 2000; Saito et al., 2002).

Mediator of Damage Checkpoint (MDC) 1

One of the first proteins being recruited to the breaks through γH2AX is the large protein MDC1 whose foci are visible already after 1 hour of repair (Lukas et al., 2004; Shang et al., 2003; Stewart et al., 2003; Stucki et al., 2005). ATM and MRN complex recruitment is dependent on MDC1, indeed MDC1-depleted cells show severe defects in ATM foci formation and MRN breaks localization; moreover mutants of NBS1 (part of the MRN complex) unable to interact with MDC1 are not recruited to damaged sites (Lukas et al., 2004). MDC1 binds γH2AX through its tandem BRCA1 C-terminal (BRCT) domain that recognizes phosphorylated proteins (Stewart et al., 2003; Stucki et al., 2005) and serves as a platform to recruit additional ATM through its Fork-Head Associated (FHA) domain. It has been shown that ATM^{s1981p} is not diminished in MDC1 deficient cells, but its recruitment is impaired (Lou et al., 2006) thus activated ATM still requires MDC1 to localize at the breaks. Recruitment of additional ATM is needed to phosphorylate additional H2AX giving rise to a positive feedback loop that will expand for megabases (Pilch et al., 2003) to amplify the signal. Moreover MDC1 is ubiquitinated on its BRCT domain (Strauss and Goldberg, 2011) and this ubiquitination is important for the downstream recruitment of Receptor Associated Protein (RAP) 80, part of the BRCA1A complex (described hereafter), fundamental for the ubiquitin signalling cascade that will follow (described hereafter), which is in turn required to spread and amplify the signal.

P53 Binding Protein (53BP) 1

53BP1 was firstly identified as p53-interacting protein in a two-hybrid screen (Iwabuchi et al., 1994) and it is one of the first proteins to co-localize with γH2AX after DSBs induction after IR exposure (Schultz et al., 2000). Mice depleted of 53BP1 have a similar phenotype to H2AX null mice (Morales et al., 2003; Ward et al., 2003). How 53BP1 is recruited at the

breaks has been matter of debate in respect to the requirement of MDC1 for this purpose (Eliezer et al., 2009; Fradet-Turcotte et al., 2013; Stewart et al., 2003; Zgheib et al., 2009), however it is clear the impact of chromatin modification on the binding and release of 53BP1. 53BP1 binding to chromatin is mediated by its tandem tudor domain that can interact with dimetylated K20 of histone H4 (H4K20me2), although this histone mark is not shown to increase after damage induction, or specifically induced at the sites of breaks, thus other mechanisms of regulation must be involved in 53BP1 binding at DSBs (Botuyan et al., 2006). Additional regulation is mediated by its C-terminal ubiquitin interacting domain, capable of binding to monoubiquitinated histone H2A on K15 (H2AK15ub) (Fradet-Turcotte et al., 2013); this modification is instead specifically induced at the sites of breaks by the Ring Finger Protein (RNF) 168 (described hereafter). Moreover, a third chromatin mark is involved in 53BP1 dynamics at the breaks; acetylation of K16 on histone H4 (H4K16ac) by Tip60 is shown to reduce the affinity of 53BP1 for H4K20me2, by creating steric hindrance that would favour the release of 53BP1 (Tang et al., 2013). Moreover 53BP1 interacts with a series of proteins that promote his release from the break in specific moments of the cell cycle. The high level of regulation of 53BP1 dynamics at the break site is probably due to its key role in the choice between different DDR pathways that will be used downstream to repair the break (described hereafter).

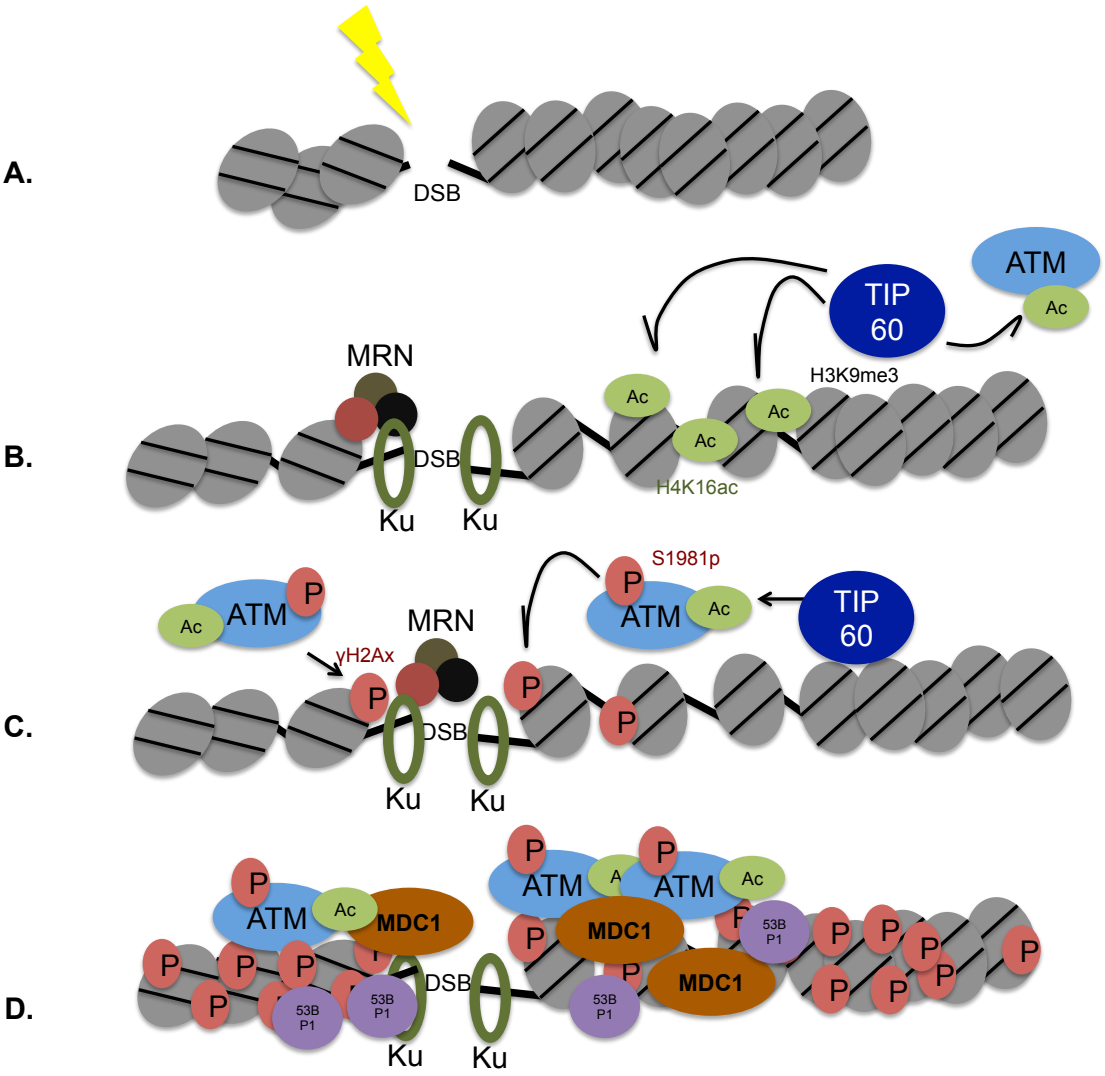

Figure 1 First steps of the DNA damage response.

(A) Double Strand Break formation. (B) After DSB induction transient chromatin relaxation is induced through acetylation by the HAT TIP60, which will also acetylate ATM kinase inducing its activation. Recruitment of TIP60 to the break requires H3K9me3 and the MRN complex. DNA ends are recognized by the Ku heterodimer (C) activation of ATM is induced by TIP60 acetylation and concomitant autophosphorylation on serine 1981; activation induced monomerization of ATM and phosphorylation of downstream substrates such as the histone variant H2AX (γH2AX). (D) γH2AX recruits downstream factor MDC1, which will in turn recruit additional ATM that will phosphorylate more H2AX, contributing to the signal spreading for megabases around the lesions and to the formation of IRIF. 53BP1 will also be recruited at γH2AX foci through its interaction with constitutive and induced histone PTMs, respectively H4K20me2 and H2AK15ub.
AC = acetylation; P = phosphorylation

1.2.1 DDR pathways

As mentioned before, after a unique signalling cascade, the choice of the pathway is made on the basis on different factors, which includes the current cell cycle stage, the chromatin environment and chromatin localization in the highly compartmentalised space of the nucleus. In addition, scheduled physiological breaks, such as in meiosis or in Immunoglobulin (Ig) Class switching, are repaired by already decided pathways, important

for the physiological outcome of the repair. The main two pathways of repair are the Non-Homologous End Joining (NHEJ), generally considered error-prone, and the error-free Homologous Recombination (HR) pathway, that are implemented, in the case of failure, by more mutagenic pathways such as Alternative-End Joining (Alt-EJ) and Single Strand Annealing (SSA). The principal difference between HR and NHEJ is the use of a sister chromatid to copy the genetic information that have been lost in the damage. HR is generally defined error-free because it uses this strategy to repair the break, thus is mainly active during S/G2 phases of the cell cycle, when a sister chromatid is available; while NHEJ is generally defined as error-prone, being active in all cell cycle phases and based on the ligation of two broken ends without using complementary information.

1.2.2 Non-Homologous End Joining (NHEJ)

DSBs that arise in the genome in all the cell cycle stages are generally repaired by the classical NHEJ, a highly conserved pathway among eukaryotes that is based on the simple re-ligation of the two broken ends. If the ends are compatible for ligation, this can happen without errors, but a minor processing of the ends can easily produce small deletions or insertions thus making NHEJ an error-prone repair pathway (Figure 2). Although NHEJ is active throughout the cell cycle is basically the only operating pathway in G1, when HR is not competing.

Break recognition: Ku heterodimer and MRN complex

As mentioned before, break recognition is one of the first steps of the DDR and it involves different proteins, among which the MRN complex and the Ku heterodimer composed of Ku70 and Ku80. Between MRN and Ku, the latter is involved in NHEJ whereas the MRN complex plays a major role in HR (described hereafter). Thus a mechanism that directs pathway choice must exist at earlier steps. In yeast as in human, Ku and MRN (MRX in yeast; Mre11, Rad50 and Xrs2) are recruited at the breaks in a simultaneous way (Wu et al., 2008) and in yeast the MRX complex is able to displace Ku to initiate HR (Shao et al., 2012). However in human cells, where Ku is very abundant and NHEJ is the major repair pathway, a simple competition between MRN and Ku is not enough to displace Ku and initiate HR (Sun et al., 2012). In fact it has recently been shown that Ku70 is phosphorylated and this phosphorylation is required for its dissociation from DSB ends. Indeed blocking the phosphorylation sites induces a significant decrease in HR mediated repair (Lee et al., 2016).

DNA-PK complex

The two broken ends are recognized by the KU heterodimer. Ku can only load at DNA ends independently on the structure of the end: it can bind to blunt ends, ends with a 5'- to 3'-overhang and hairpin ends (Mimori and Hardin, 1986). Moreover multiple Ku molecules can bind to a DNA end through a binding and translocation mechanism (Paillard and Strauss, 1991). The association of Ku heterodimer to both the ends was shown to limit DSB mobility, indeed Ku depletion leads to separation of the two broken DNA ends and increase the translocation frequency (Roukos et al., 2013; Soutoglou et al., 2007). After Ku heterodimers associates with the broken ends DNA-PKcs is recruited. DNA-PKcs is one of the largest polypeptide in human cells and it is recruited at the break in a Ku-dependent manner (Gottlieb and Jackson, 1993; Taccioli et al., 1994). Ku and DNA-PKcs form the so-called DNA-PK complex together with DNA, and no interaction between Ku and DNA-PKcs is observed in absence of DNA (Suwa et al., 1994). Mammalian cells deficient for Ku or DNA-PKcs are more sensitive to irradiation (Boubnov et al., 1995; Getts and Stamato, 1994; Peterson et al., 1995; Taccioli et al., 1994) although this can vary accordingly to the cell cycle (Lee et al., 1997). Once the complex is formed, DNA-PKcs acquires serine/threonine kinase activity and its first target seems to be itself with more than 15 autophosphorylation sites.

Ends processing

Most DSBs have incompatible ends that preclude direct ligation thus nuclease activity is required to ensure that the two ends are compatible. When little resection happens in NHEJ errors can be produced due to the absence of the homologous chromatid for repair. The DNA-PK complex undergoes autophosphorylation and recruits the endonuclease Artemis that can digest ssDNA and dsDNA and has both 5' and 3' overhang endonuclease activiy when in complex with the DNA-PK complex (Chang and Lieber, 2016; Chang et al., 2015). It has been estimated that 20-50% of the DSBs that are produced by IR require Artemis for repair (Kurosawa et al., 2008; Riballo et al., 2004). The remaining amount of breaks can be resected by additional nucleases (Kanno et al., 2007; Pannunzio et al., 2014) or have a favourable DNA end configuration for ligation without processing. Once the DNA has undergone resection, polymerases will act to add the necessary nucleotides to have as little as 4 base pairs (bp) of homology to direct the subsequent ligation. Polymerases (Pol) μ and Pol λ can add nucleotides in a template-independent manner (Bertocci et al., 2006)

Ends Ligation

Ligation of the two ends, after processing, requires the DNA ligase IV and X-ray repair cross-complementing protein 4 (XRCC4) in eukaryotes, the most important proteins for NHEJ. In the presence of Ku the XRCC4-DNA ligase IV complex can ligate, with a 10-fold increased efficiency, ends that possess 2 bp of homology (Gu et al., 2007a) or even incompatible ends at a low efficiency (Gu et al., 2007a). When DNA ends do not share any homology or they do not possess any overhang (blunt ends) probably the addition of one more protein, the XRCC4-like factor (XLF), can increase the efficiency of re-joining (Gu et al., 2007b; Tsai et al., 2007). XLF seems to be important for the re-joining of incompatible ends that might arise often, considering that patients lacking XLF show IR sensitivity (Ahnesorg et al., 2006; Buck et al., 2006)

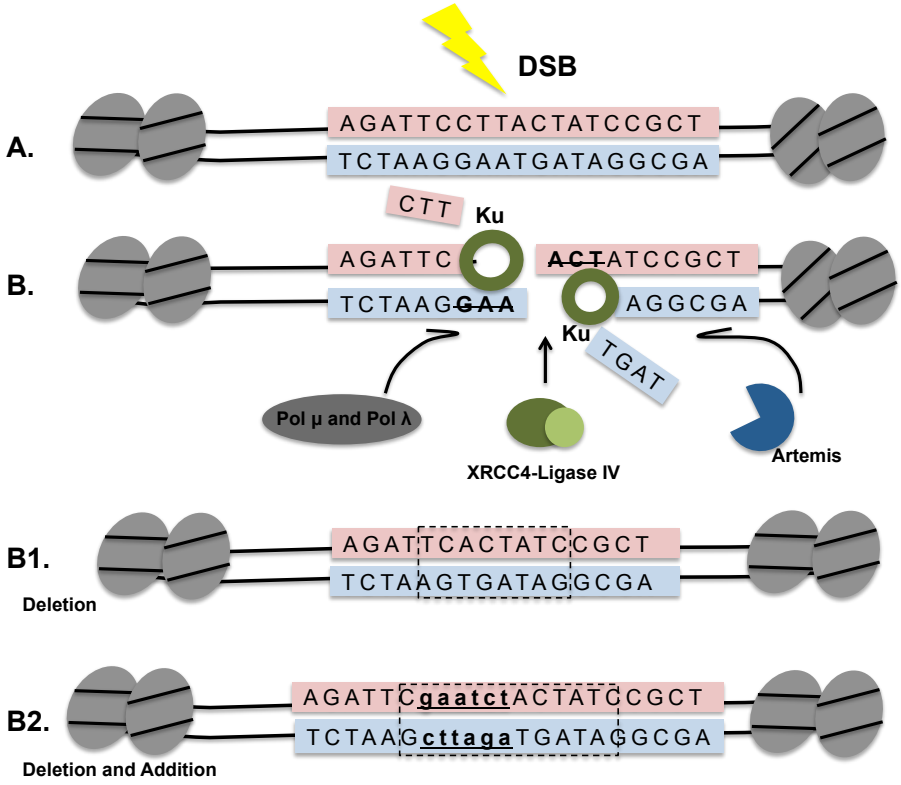

Figure 2 Non-Homologous End Joining outcomes.

NHEJ pathway does not rely on the utilization of homologous sequences to copy the missing information and it usually re-ligates the two broken ends. DSB ends might not be compatible for direct re-ligation so they undergo a process of digestion (resection) creating deletions (B1). Eventually some nucleotides might be added in a template-independent manner by specific polymerases to create few base pair homology that will favour ligation, creating deletion in the original sequence and insertion of a new sequence (B2). Adapted from (Chang et al., 2017)

1.2.3 Homologous Recombination (HR)

HR is a highly conserved pathway that relies on the same basic principles from bacteria to human. The first suggestion that recombination could be used to repair broken DNA came from studies of irradiation in yeast during the 70s (Resnick, 1976). Later on it was proposed a model for repair of restriction-enzyme cut plasmids, which involved recombination and insertion of the plasmid in the yeast genome at the locus corresponding to the homologous sequence to the cut region in the plasmid (Orr-Weaver et al., 1981). The key step of HR is the digestion of the edge of the DSB to form a 3' overhang ssDNA in a process called resection. The 3' strand will invade the homologous duplex of DNA to form a displacement loop (D-loop) and it will be used as a template to prime synthesis to recreate new DNA (Szostak et al., 1983). After this moment different models have been proposed for the resolution of the structure (Figure 3). In the Double Strand Break Repair (DSBR) model the 3' overhang of the other side of the break anneals to the displaced strand of the homologous duplex used for invasion, and will start a new synthesis that will end up ligating the 5' end of the other break (Figure 3 B). The structure formed at this point is a double-cross structure called double Holliday Junction (dHJ) that can be resolved in different ways. In one way the two "crosses" migrate toward each other and they are dissolved with the help of helicases and topoisomerases: in this way there is no exchange of genetic material and the result is called Non Crossover (NCO)(Figure 3 B1). In the second way the dHJ is resolved by nucleases, and even in this case accordingly to the position of the cut the result can be a NCO or eventually a crossover (CO) with exchange of genetic information (Figure 3 B2). NCO events can also be explained by Synthesis Dependent Strand Annealing (SDSA) model (Figure 3 B3) in which the invading 3' overhang is displaced after a limited DNA synthesis, and reanneals with the other single strand of the break. Models depicting NCO events are generally used to explain mitotic recombination, in which there is a low frequency of genetic exchange compared to meiotic recombination (Ferguson and Holloman, 1996; Nassif and Engels, 1993; Paques et al., 1998) (Figure 3).

HR is usually defined as an error-free pathway, because it uses the homologous template to faithfully replace the lost genetic information. Nevertheless highly mutagenic pathways exist that involve at least the first steps of HR. Break Induced Replication (BIR) (Figure 3A) involves invasion of the 3'overhang and subsequent start of replication from that end that will result in the transfer of genetic information from the donor causing loss of genetic material (Kraus et al., 2001; Llorente et al., 2008).

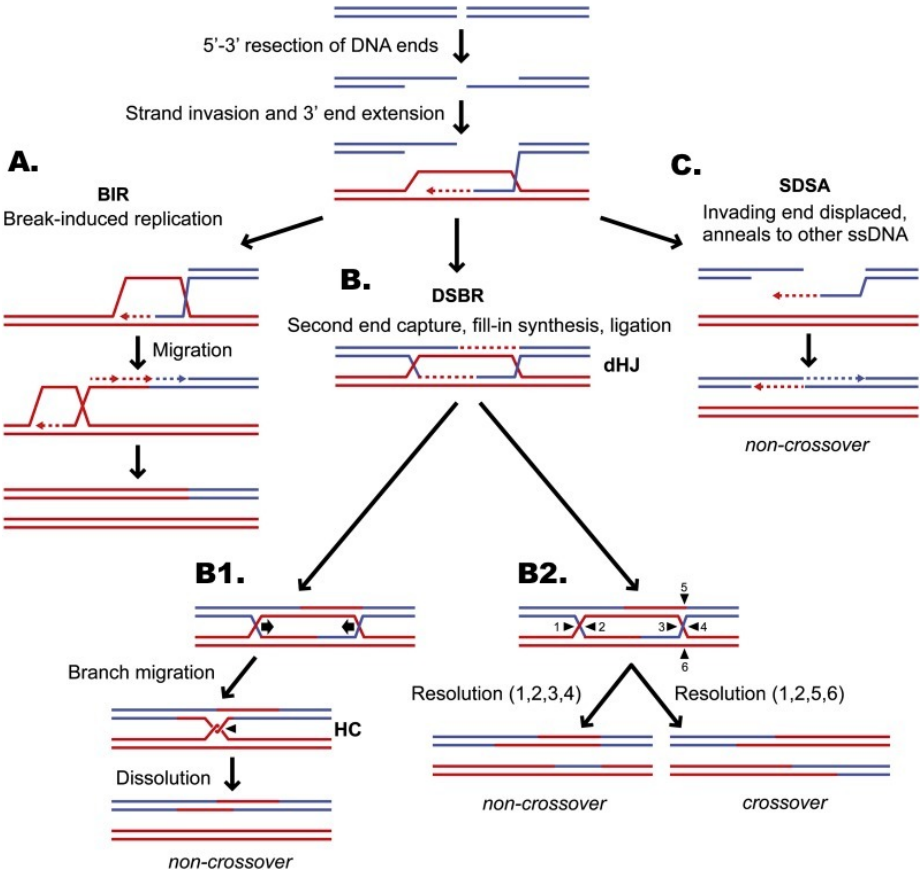

Figure 3 Different DSBs resolution pathways involving recombination events.

The resection process will digest the 5' end of the damaged DNA (blue lines) to leave a 3' overhang that can invade the homologous duplex (red lines). (A) One ended break is repaired with the BIR pathway, in which the 3' overhang invades the homologous duplex and a process of replication starts. (B) The classical model for DSB repair involves the formation of dHJ than can be resolved either by dissolution leading to NCO events, or by resolution leading eventually to exchange of genetic material in the meiotic recombination (CO events). (C) SDSA model explains also NCO events that are the most frequent in mitotic recombination. Adapted from (Symington et al., 2014).

As mentioned before the key step in HR is resection. This process requires many different proteins that will be summarized in the following sections:

MRN complex: resection machinery

The MRN complex is a dynamic machine that acts in the first steps of the DDR, being one of the first complex recognizing the breaks and fundamental for the resection process. RAD50 is the biggest subunits of the complex harbouring ATPase activity and a zinc-hook domain that drives the dimerization of RAD50 that is thus present as a dimer in the complex (Cahill and Carney, 2007; Hopfner et al., 2002). MRE11 protein, as RAD50, is also a dimer and harbours nuclease activity that is necessary for resection (Hopfner et al., 2001). Interestingly, resection is aimed to produce a 3' ssDNA overhang, but MRE11 possesses a 3'-to-5' dsDNA exonuclease activity and ssDNA endonuclease activity

(Hopfner et al., 2001). The model proposes that MRE11, after recognizing the break would move 100-200 bp from the DNA end (Shibata et al., 2014). At that point the first activity of MRE11, stimulated by CtBP-Interacting Protein (CtIP; described after) involves the catalysis of a nick in the dsDNA close to the damage site, through its endonuclease activity, (Cannavo and Cejka, 2014) and then the 3'-to-5' exonuclease activity digests the DNA towards the break (Cannavo and Cejka, 2014; Garcia et al., 2011). The other side of the nick will be digested by the simultaneous action of the helicase BLOOM (BLM), the Exonuclease (EXO) 1 together with the helicase DNA2 inducing long-range resection that will create a long ssDNA stretch of more than 1000 bp, that will be covered by RPA, needed for HR (Gravel et al., 2008; Mimitou and Symington, 2008; Zhu et al., 2008) (Figure 2). The last subunit, NBS1, is an adaptor subunit also present in a dimer in the MRN complex (Schiller et al., 2012) and is responsible for the interaction of MRN complex with other proteins, that is in turn necessary for its dynamic activation and controlled action (Lee and Paull, 2005; Limbo et al., 2012; Rahal et al., 2010).

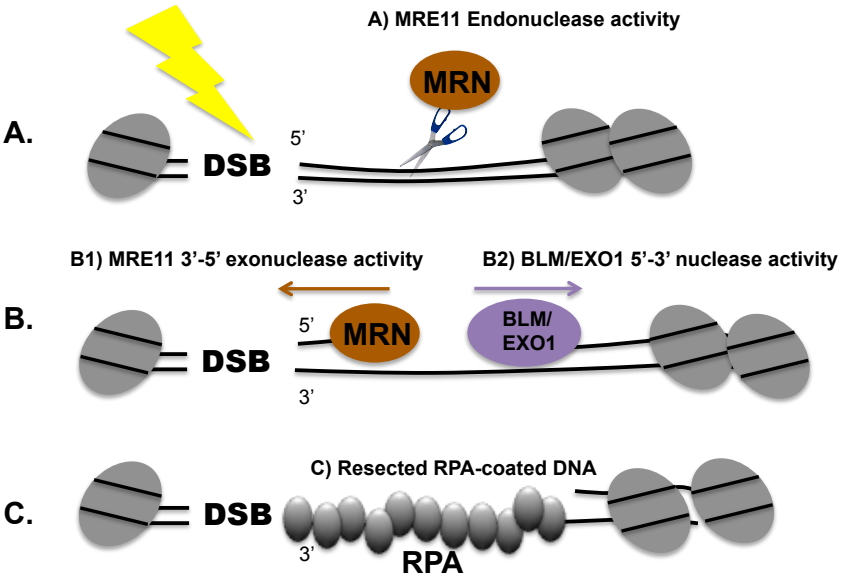

Figure 4 MRN and BLM/EXO1 in resection.

The concomitant action of MRN complex and BLM/EXO1 creates a long ssDNA filament that will be used to invade the homologous duplex of DNA in the process of HR. MRN contains MRE11, RAD50 and NBS1. (A) MRE11 possesses endonuclease and a 3'-to-5' exonuclease activity that are important in the first moments of resection, to digest a short ssDNA filament. (B) the long-range resection is instead performed by BLM and EXO1 that posses 5'-to-3' exonuclease activity. (C) RPA has high affinity for ssDNA and it fastly covers the resected DNA

CtIP; a commitment for resection and HR

CtIP is the principal regulator of the choice between HR and NHEJ, and the first initiator of resection. Interestingly short interfering (si) RNAs depletion of CtIP does not confer DSB

repair defects in G2 phase of the cell cycle; on the contrary it has been observed that repair even happens with a faster kinetics in G2 in the absence of CtIP and it is impaired only upon additional abolishment of NHEJ (Shibata et al., 2011). At the same time depletion of downstream factors confers DSB repair defects in G2 that are rescued by concomitant depletion of CtIP (Kakarougkas et al., 2013). These results suggest that in the absence of CtIP resection is not initiated and eventually the damage can be repaired by NHEJ, but once resection has started NHEJ fails to repair the break. CtIP harbours a 5' endonuclease catalytic activity that acts on branched DNA structures (Makharashvili et al., 2014); however this activity is dispensable for its function in resection (Makharashvili et al., 2014). CtIP best known function is its interaction with Breast Cancer (BRCA) 1 and the MRN complex that is required to stimulate the initial stages of resection. The specific stimulation of resection only in S/G2 phases depends on the activity of Cycline Dependent Kinases (CDKs) that phosphorylate CtIP (Aylon et al., 2004; Huertas et al., 2008; Ira et al., 2004; Jazayeri et al., 2006); CtIP phosphorylation is required for its interaction with BRCA1 and thus for its recruitment at the breaks and subsequent stimulation of MRN nuclease activity (Chen et al., 2008; Huertas and Jackson, 2009; Yu and Chen, 2004; Yun and Hiom, 2009). Moreover CtIP levels are higher in S/G2 compared to G1 (Chen et al., 2008) and this could represent an additional level of cell cycle specific regulation. As mentioned before 53BP1 protein dynamic at the breaks is highly regulated; phosphorylation of CtIP by CDKs and its subsequent association with BRCA1 and MRN complex has also the role of displacing 53BP1 from DSB ends, to favour resection (Chen et al., 2008). The exact mechanism of the displacement is not clear yet, but is has been shown that loss of BRCA1 induces 53BP1 recruitment in G2 phase and *vice versa* loss of 53BP1 induces ectopic recruitment of BRCA1 in G1 (Escribano-Diaz et al., 2013). Moreover depletion of 53BP1 restores resection in cells lacking BRCA1 (Bouwman et al., 2010; Bunting et al., 2010; Cao et al., 2009) but not in cells lacking CtIP, consistent with the additional more active role of CtIP in actively promoting resection through MRE11 activation.

BRCA1: multiple interactions playing in resection

BRCA1 is probably one of the master regulator of DNA damage repair due to its capability in forming different complexes that can play different and opposite functions during HR (Table 1). BRCA1 protein contains a Really Interesting New Gene (RING) domain that mediates its interaction with BRCA1 Associated RING domain protein (BARD) 1, a BRCT domain through which it can interact mutually exclusively with different proteins (Huen et al., 2010) and it harbours an E3 ubiquitin ligase activity that plays a major role during the

DDR (described after) (Brzovic et al., 2006; Christensen et al., 2007; Morris and Solomon, 2004; Nishikawa et al., 2004; Starita and Parvin, 2006). BRCA1 most widely accepted role is that of promoting HR by favouring end resection (Moynahan et al., 1999; Schlegel et al., 2006). The BRCA1 C complex is composed of CtIP and the MRN complex thus is automatic the connection with DNA end resection. BRCA1 BRCT domain can interact with the phosphorylated form of CtIP arising during S/G2 phases (Yu and Chen, 2004) and it might act as a scaffold to stabilize MRN-CtIP. Indeed a phosphorylation-defective mutant of CtIP which could not interact with BRCA1 was shown to have a reduced formation of ssDNA at the damaged site and deficiency in HR mediated repair (Yun and Hiom, 2009). A more recent study suggested that BRCA1 might also be involved in the dephosphorylation-dependent displacement of 53BP1 from the broken ends, thus favouring HR and abrogating NHEJ (Isono et al., 2017). A first role of BRCA1 in HR was already postulated many years ago through its interaction with Breast Cancer (BRCA) 2 that had already a well-established role in HR through its interaction with RAD51 (Bhattacharyya et al., 2000; Chen et al., 1998; Scully et al., 1997; Thorslund and West, 2007; Xia et al., 2006). Differently from BRCA1 C complex, the interaction between BRCA1 and BRCA2 is required at later steps, when resection has produced long stretches of ssDNA that is covered by RPA. At this step, BRCA1, BRCA2 and Partner and Localizer of BRCA2 (PALB2) are needed to load RAD51 onto the ssDNA and displace RPA. Thus accumulation of BRCA2 and RAD51 at damage foci is dependent on BRCA1 although it is not the only responsible, because PALB2 mutants that cannot interact with BRCA1 are still able to accumulate at DSBs (Sy et al., 2009).

Surprisingly BRCA1 roles in HR seem to be more complex. In fact the BRCA1 A complex, in addition to its best known role in the G2/M checkpoint (Table 1) (Yarden et al., 2002), has been shown to have a role in resection different from the canonical. Indeed BRCA1 A inhibits HR by restricting resection (Coleman and Greenberg, 2011). Depletion of RAP80 increases resection specifically in S/G2 phases, increases HR and SSA efficiency and increases Sister Chromatid Exchange (SCE) that only happens as a result of HR (Coleman and Greenberg, 2011). The increased resection in RAP80 deficient cells is probably dependent on a shift of BRCA1 from the A complex to the C complex that stimulates resection (Coleman and Greenberg, 2011).

BRCA1 complex	Function	Components	Reference
Core complex	Promotes E3 ubiquitin ligase activity	BRCA1-BARD1	(Wu et al., 1996)
BRCA1A	Promotes G2/M checkpoint. **Inhibits HR by restricting resection in S/G2**	BRCA1-Abraxas-RAP80-BRCC36-BRE-MERIT40	(Coleman and Greenberg, 2011; Yarden et al., 2002)
BRCA1B	DNA replication and S phase progression	BRCA1-BACH1-TOPBP1	(Cantor et al., 2001)
BRCA1C	**Promotes HR in S/G2 by stimulating initial step of resection through MRN nuclease activity**	BRCA1-CtIP-MRN complex	(Chen et al., 1998; Yu et al., 1998; Yun and Hiom, 2009)
BRRC	**Promotes HR by facilitating loading of RAD51 at ssDNA**	BRCA1-BRCA2-PALB2-RAD51-BRCC36-BRE	(Dong et al., 2003; Sy et al., 2009; Zhang et al., 2009a; Zhang et al., 2009b)

Table 1 Different BRCA1-containing complexes.

Table representing the different known complexes containing BRCA1. In bold are highlighted the complexes that have a recognized role in resection. Adapted from (Huen et al., 2010)

RPA, Rad51 and BRCA2: playing on ssDNA

RAD51 is a central player in HR, needed for the actual process of recombination, presynapsis, synapsis and post-synapsis formation (Sung et al., 2003). After a long strech of ssDNA is produced by the concomitant action of MRN complex and BLM/exo1 nucleases, it will be covered at a first moment by RPA. Chromatin Immunoprecipitation (ChIP) analyses and cytology data have shown that RPA is the first protein arriving at DSBs after resection has occurred (Gasior et al., 1998; Wolner et al., 2003) given its high affinity for ssDNA. However the presence of RPA on ssDNA blocks the actual recombination process, and for recombination to proceed RAD51 must replace RPA on the filament. Mutations in RPA that inhibit HR by slowing down this process and inhibiting RAD51 loading have been reported (Kantake et al., 2003). However RPA has also functions in promoting HR by removing secondary structures on ssDNA that could impede RAD51 loading (Sung et al., 2003). Moreover RPA protects the 3' overhang from digestion and the filament ssDNA-RPA is sensed by the checkpoint kinase ATRIP allowing enough time to repair through cell cycle arrest (Ball et al., 2007; Choi et al., 2010; Zou and Elledge, 2003). PTMs of RPA also have been shown to play a major role in HR; phosphorylation of RPA by ATM and Cell cycle Dependent Kinase (CDK) 1 is critical for RAD51 recruitment (Shi et al., 2010; Zou and Elledge, 2003). RAD51 recruitment is helped

by a family of five proteins (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3), called RAD51 paralogs (Thacker, 2005) and deficiency in RAD51 paralogs abrogates RAD51 foci formations in response to IR (Takata et al., 2001). In addition, RAD51 loading on the ssDNA is accompanied by a series of proteins called RAD51 regulators. One of the main regulators in mammals is BRCA2 (in yeast Rad52 helps the loading of RAD51, but no homology exists between Rad52 and BRCA2) that helps the formation of RAD51 nucleoprotein filaments both *in vitro* and *in vivo* (San Filippo et al., 2008; Thorslund and West, 2007). BRCA2 interaction with RAD51 is mediated by BRC domains in BRCA2, that target RAD51 to ssDNA stabilizing the interaction RAD51-ssDNA by down-regulating RAD51 ATP hydrolysis, and it also prevents the nucleation of RAD51 on dsDNA (Carreira et al., 2009; Carreira and Kowalczykowski, 2011; Pellegrini et al., 2002; Shivji et al., 2009). Moreover BRCA2 can interact with RAD51 through its C-terminal domain, in a cell cycle dependent manner and only with the nucleofilament form of RAD51; this binding is required to stabilize RAD51 at replication forks and is not essential for HR mediated repair (Ayoub et al., 2009; Davies and Pellegrini, 2007; Esashi et al., 2007). The filament ssDNA/RAD51 is stimulated to invade the homologous dsDNA by the translocase RAD54, and once in the duplex it will scan for homology (Heyer et al., 2006; Petukhova et al., 1998). It has been shown in yeast that 8 nucleotides of homology are the minimum requirement for dsDNA sampling and after 15 nucleotides of homology RAD51 is no longer able to scan more dsDNA and this represent a “HR commitment” (Qi et al., 2015). The nucleotide exchange activity of RAD51 happens in three nucleotides step (Qi et al., 2015). In yeast the disassembly of RAD51 requires the helicase Srs2, which interacts directly with RAD51 to stimulate its ATP hydrolysis activity and reduce its affinity for ssDNA (Antony et al., 2009). Similar function in humans can be played by the helicases RecQ5, BLM and FANCJ (Bugreev et al., 2007; Hu et al., 2007; Sommers et al., 2009). Resolution of the D-loop can lead to CO or NCO products, as depicted in Figure 3. This process will require the concomitant action of resolvases, helicases and topoisomerases such BLM and TOP3α (Fricke and Brill, 2003; Schwartz and Heyer, 2011).

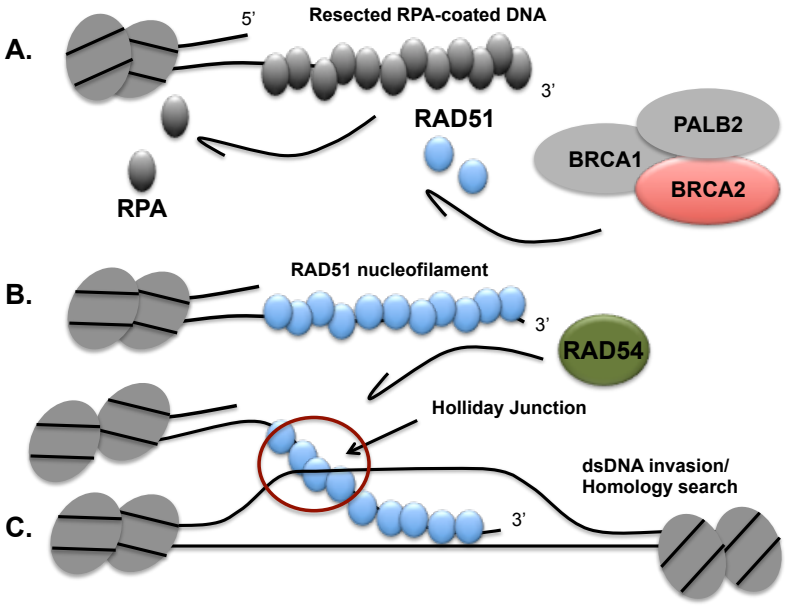

Figure 5 RAD51 loading on resected DNA

(A) After resection the exposed strand will be immediately covered by Replication Protein A (RPA) that has a very high affinity for ssDNA and it protects the 3' end from digestion. To allow strand invasion and recombination RPA must be replaced by RAD51. A simple competition is not a likely scenario given the high affinity of RPA for ssDNA. (B-C) RAD51 loading is regulated by RAD51-regulators, such as BRCA2 and PALB2, that increase the affinity of RAD51 for ssDNA and RAD51 loading. (D) The invasion of the duplex to scan for homology will take place with subsequent formation of a Displacement (D)-loop and Holliday Junction (HJ). These branched structures will be resolved as depicted in Figure 3

1.2.4 Single Strand Annealing (SSA)

SSA is a highly mutagenic pathway that relies on resection at least in the first steps sharing the resection machinery with HR. SSA takes place when resection is enough to reveal a complementary sequence between the two ssDNA and the annealing between them will eventually displace the sequence in between if unique (Figure 6). SSA can be responsible for the spontaneous deletion between direct repeats. SSA shares only the first resection steps with the HR pathway; it does not rely on homologous duplex of DNA thus it does not require RAD51 coated filament to initiate strand invasion (Sung, 1997). Resection depends on CtIP and the ssDNA will be coated by RPA but after resection has exposed the two complementary sequences these will anneal in a process dependent on RAD52 (Bhargava et al., 2016). Cleavage of the overhangs that are displaced by the annealing will be mediated by XRCC1 and XPF nucleases, while which polymerases and ligases are involved in the process of SSA is still unknown.

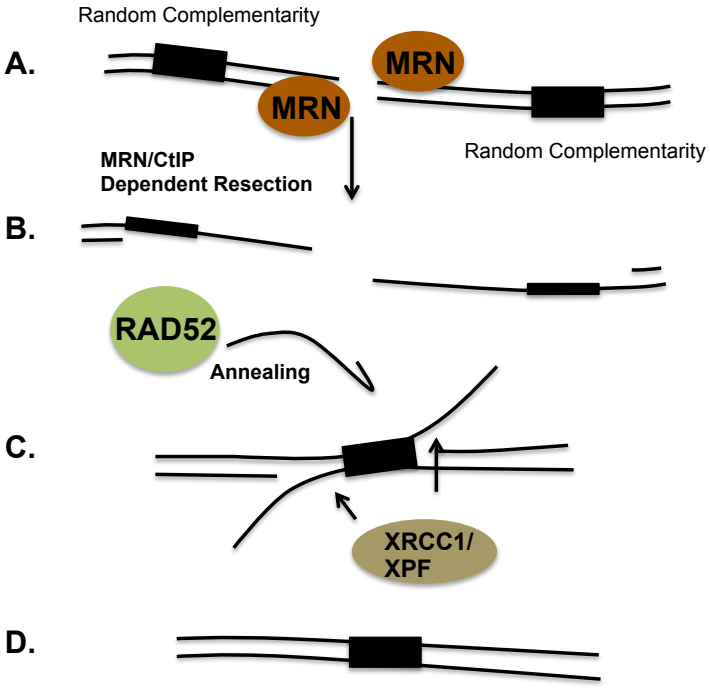

Figure 6 Schematic representation of the Single Strand Annealing (SSA) pathway.

(A) the MRN complex is involved in the first resection steps, as in the HR pathway. (B-C-D) When resection has exposed the complementarity, the two segments will anneal in a process dependent on RAD52. The overhang segments will be removed by XRC1/XPF nucleases.

1.2.5 Alternative End Joining (Alt-EJ)

Alt-EJ is a highly mutagenic pathway that takes place mostly when classical NHEJ is inactive. Indeed yeast mutants of Ku80 displayed a residual end joining activity that resulted in recombination between short repetitive elements generating deletions (Boulton and Jackson, 1996). This pathway, existing also in human cells (Della-Maria et al., 2011; Wang et al., 2003) was found to use short homology segments at a higher rate compared to NHEJ.

Break recognition: a PARylation-dependent mechanism

Recognition of a DSB end is usually accomplished by the Ku heterodimer or alternatively by the MRN complex accordingly to the cell cycle stage and the pathway choice. Recognition of the break has been also shown to be a process dependent on addition of Polymers of ADP-ribose on proteins, a process accomplished by PARP enzymes and known as PARylation. PARylation has a being shown to be one of the earliest events in the DDR signalling cascade, quickly removed by PAR glycohydrolases (PARG) (Beck et al., 2014b; Gagne et al., 2006; Haince et al., 2008; Hakme et al., 2008). Among the different PARP enzymes until now only PARP1, PARP2 and PARP3 have been shown to be involved in the DDR (Beck et al., 2014b; Hakme et al., 2008). PARP1 and PARP2 are

immediately detected at IRIF (Tartier et al., 2003). The role of PARP enzymes in Alt-EJ is undertaken by PARP1. Different reports have shown that depletion of PARP1 or its chemical inhibition significantly decreases DSB end joining in cells lacking Ku70 or Ku80 (Wang et al., 2006). It has also been shown that PARP1 competes with Ku70/80 for ends binding (Paddock et al., 2011; Wang et al., 2006) inhibiting classical NHEJ and stimulating the highly mutagenic Alt-EJ pathway, important in Class Switch Recombination (CSR) (Robert et al., 2009). A direct competition between PARP1 and Ku and the fact that Ku inhibits PARP1 recruitment at breaks suggests that the choice between NHEJ and Alt-EJ is mainly dependent on Ku presence. In addition to their well- established role in ALT-EJ, PARP enzymes have been found to be involved also in HR and classical NHEJ. In fact PARP1 is involved in HR at stalled or collapsed replication forks (Bryant et al., 2009) while PARP3 favours NHEJ by limiting extensive resection mediated by CtIP/MRN complex and helps to recruit/stabilize Ku80 at the DSBs (Beck et al., 2014a).

Resection, processing and ligation in Alt-EJ

Alt-EJ is a highly mutagenic pathway that, differently from NHEJ and similarly to HR, relies on MRN-dependent resection. The initial resection machinery is shared between HR and Alt-EJ, using in both cases the endonuclease activity of MRE11 and CtIP (Deriano et al., 2009; Lee-Theilen et al., 2011; Truong et al., 2013; Xie et al., 2009; Zhang and Jasin, 2011). However only short-range resection is utilized by the Alt-EJ pathway indeed recruitment of BLM/EXO1 by the MRN complex stimulates HR (Truong et al., 2013). The endonuclease activity of MRE11 generates 15-100 bp nucleotide 3’ overhangs that can be annealed and stabilized by Polθ, even if harbouring only 2 bp of homology (micro-homology aka MH) (Wyatt et al., 2016). Polθ was found to be involved in the random insertions found after deleterious chromosome end-to-end fusion due to telomeres de-protection in mammalian cells, and absence of Polθ in *Drosophila* cells has been shown to leave large deletions at the breaks site (Chan et al., 2010; Wyatt et al., 2016). After annealing Polθ extends the 3’ end using the annealed strand as a template and the resulting dsDNA can be sealed by DNA ligase I (Lig1) and ligase III (Lig3) together with XRCC1 (Audebert et al., 2004; Liang et al., 2008; Wang et al., 2005).

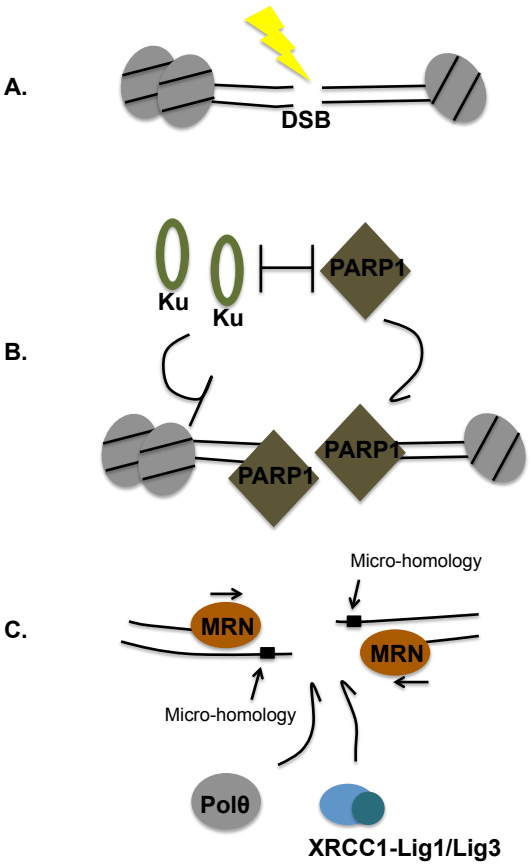

Figure 7 Schematic representation of Alternative End Joining (Alt-EJ) pathway

(A-B) Break recognition involves a competition between Ku proteins and PARP1. (C) The first steps of resection are accomplished by the MRN complex, that resects for short range distance until it reveals a short homology. Polθ is involved in inserting random nucleotides, and the gap will be filled by Lig I, Lig III and XRCC1.

1.3 Post Translational Modifications (PTMs) in the DDR

The cell response to DNA damage relies on the effective recruitment of DDR factors on chromatin around the lesion, thus histone PTMs play a fundamental role in this process. The recognition of the DNA damage is orchestrated by the activation of a signalling cascade that finally activates the desired repair pathways. The most studied PTM of proteins involved in the DDR, not only histones, is phosphorylation and dephosphorylation. However it has become clear that the proper repair relies on a plethora of different modifications. Moreover it is recognized that not only the modification *per se*, but also the crosstalk and co-existence of different modifications is of vital importance for the maintenance of genome integrity in response to damage. For this reason a single modification must be considered in a complex network of PTMs that all stimulate formation and/or removal of each other.

1.3.1 Ubiquitination and Deubiquitination of Histone proteins in the DDR

Ubiquitination consists in the enzymatic reaction of ligating the 8 kDa ubiquitin protein, onto the lysine of an acceptor protein. Protein ubiquitination requires the coordinated action of three enzymes: an ubiquitin-activating enzyme E1, an ubiquitin-conjugating enzyme E2 and an ubiquitin ligase E3. Accordingly to how many ubiquitin molecules are linked in chain to the acceptor protein, ubiquitination varies between mono and poly-ubiquitination, with the latter also having different outcomes depending on the type of link between one ubiquitin molecule and the other. For this reason people refer to these modifications as an "ubiquitin code" that, although still not fully understood, relies on basic principles. For examples chains formed by ubiquitin molecules connected through their lysines at position 48 (K48-ubiquitin chains) are generally coding signals for proteasomal degradation (Hershko and Ciechanover, 1998). In contrast, ubiquitin chains linked by their lysine at position 63 (K63-ubiquitin chains) have exclusively non-proteolytic functions (Zhao et al., 2007).

In the context of DSBs, ubiquitination happens in many regulatory steps of the DDR, both on histones and non-histone proteins.

Ubiquitination of histone proteins in the DDR

Ubiquitination of histone proteins in response to DSBs mainly happens on histones H2A and H2B (Figure 8). Specific ubiquitinating enzymes for both histones have been shown to play a role in DSB repair. Early studies found a role for the E3 ubiquitin ligase RNF8 in establishing an ubiquitination cascade that is directly linked to γH2AX (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). H2AX is the first histone being modified in response to DSBs and, as mentioned before, is phosphorylated on serine 139 (Rogakou et al., 1998). RNF8 contains a RING domain (required for the conjugation of ubiquitin with its substrate) and a FHA domain that is required for its interaction with ATM mediating RNF8 interaction with phosphorylated MDC1, which itself selectively binds γH2AX (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). The ubiquitination activity of RNF8 is required for recruitment of downstream effectors of DDR; in fact it has been shown that mutation of either the RING domain, or the FHA domain affects the recruitment of BRCA1 and 53BP1 (Huen et al., 2007; Mailand et al., 2007). *In vitro* studies suggested that RNF8 was capable of ubiquitinate both H2A and H2AX and RNF8 depletion has been shown to impair the DNA-damage-dependent ubiquitination of these two histones (Mailand et al., 2007); however it seems that RNF8 is uncapable of acting on nucleosomal H2A (Mattiroli et al., 2012). Indeed another E3 ubiquitin ligase is responsible for H2A ubiquitination;

RNF168 (Doil et al., 2009; Stewart et al., 2009). It has been suggested that RNF168 is recruited at the site of breaks by the ubiquitination action of RNF8 on surrounding chromatin, specifically on linker histone H1 (Thorslund et al., 2015). Once recruited, RNF168 is required for monoubiquitination of N-terminal tail K13 and K15 of histone H2A and for the formation of K63-ubiquitin chains, for whose extension it still requires the presence of RNF8 (Gatti et al., 2012; Mattiroli et al., 2012). Concerning the monoubiquitination of H2A it has recently been shown that 53BP1 might directly recognize H2AK15ub with its ubiquitin dependent recruitment (UDR) domain, in addition to its capability to recognize H4K20me2 by its tandem TUDOR domain (Fradet-Turcotte et al., 2013). Moreover it has been shown that binding to both H2AK15ub and H4K20me2 is necessary for the formation of 53BP1 IRIF suggesting cooperativity between these two histone PTMs in 53BP1 binding to chromatin (Fradet-Turcotte et al., 2013). The cryo-electron microscopy (cryo-EM) structure of a dimer of 53BP1 binding to chromatin elucidated the nature of the interaction and the specificity for H2AK15ub over K13ub (Wilson et al., 2016). Monoubiquitination of H2A on K15 and subsequent recruitment of 53BP1 plays a role in promoting NHEJ, because presence of 53BP1 counteracts resection. As mentioned before RNF168 is also involved in the extension of the ubiquitin mark at the site of breaks, forming K63-linked ubiquitin chains involved in the recruitment of downstream factors. One the first factors recruited is the BRCA1-A complex (Sobhian et al., 2007), composed of BRCA1/BARD1 complex, RAP80, MERIT40, Abraxas and the DUB BRCC36. The recruitment of this complex has been shown to limit resection to impede hyperactive HR (Hu et al., 2011; Savage and Harkin, 2015). RAP80 is responsible for the recruitment of the complex through its ubiquitin interacting motif (UIM) and BRCC36 is required for the cleavage of K63 ubiquitin chain, a step that is fundamental to limit resection (Ng et al., 2016), while the role of BRCA1 in this context is not elucidated yet. The complex BRCA1/BARD1 (in the BRCA1-A complex) is an E3 ubiquitin ligase, whose substrate has been recently identified to be K127 and K129 again on histone H2A (Kalb et al., 2014). This ubiquitination was found to promote resection and thus HR (Densham et al., 2016) creating confusion around BRCA1 role in resection (see Table 1). One elucidation might come to the different localization of the different complexes respect to the break. It has been shown that BRCA1-A complex with RAP80, is found several kilobases away from the break, where it seems to inhibit resection; while a second BRCA1-containing complex (BRCA1-C complex) is found at the vicinity of the break (Coleman and Greenberg, 2011) where it seems indeed to have the opposite function

stimulating resection, together with CtIP and the MRN complex. However the recruitment of BRCA1-C is dependent on H3K9me2, rather than RNF168 dependent ubiquitination (Wu et al., 2015) and the function of BRCA1 ubiquitination activity in this pathway is still unclear. Ubiquitination of H2A happens also on K199 which, differently from K13 and K15, is found inside the core domain of H2A; this modification is generally associated with transcription repression (Wang et al., 2004; Zhou et al., 2008; Fang et al., 2004) and happens via the E3 ubiquitin ligase Ring2, while RNF168 activity is specific for K13/15 (Gatti et al., 2012; Mattiroli et al., 2012). The physiological role of H2AK199ub in the DNA damage response is not fully understood. It has been suggested that it might favour NHEJ, and that it could shield the DSB site from intrusion of the transcription machinery.

Monoubiquitination of Histone H2B happens, in mammals, on the K120; The discovery of H2Bub1 was in 1980 when the Bonner group discovered that human and mouse cells ubiquitinate 1-1,5% of their total histone H2B (West and Bonner, 1980) but it was only in 2000 that the E2 and E3 enzymes responsible for this ubiquitination were discovered in yeast, as respectively Rad6 and Bre1 (Hwang et al., 2003; Robzyk et al., 2000; Wood et al., 2003). The discovery of Bre1 in yeast, that ubiquitinates K123 on H2B leads to the subsequent discovery of the mammalian homologs RNF20/RNF40 that ubiquitinate K120 (Zhu et al., 2005). Early reports in yeast connected for the first time ubiquitination of H2B with DNA damage showing that H2Bub1 is required for genotoxic stress checkpoint activation (Giannattasio et al., 2005). The activation of the checkpoint is required to control cell cycle progression in response to stress and in normal yeast cells after genotoxic stress is required to delay cell cycle progression. It was shown that bre1Δ cells and cells expressing H2B point mutants with conversion from K to the non-ubiquitinable Arginine (R) in position 123 (H2BubK123R) have a deficient G1-S and intra-S DNA damage checkpoint, meaning that they proceed normally in cell cycle progression, even in the presence of DNA damage (Giannattasio et al., 2005). Moreover it was suggested that this is due to the stimulation, by H2Bub1, of the methylation of K4 and K79 of Histone 3 (H3K4me and H3K79me) that happens on certain promoters. Indeed the same phenotype of bre1Δ cells was observed in cells depleted of the methylase Dot1 involved in methylation of H3K4 (Giannattasio et al., 2005). In mammals, the heterodimer RNF20/RNF40 was found to associated with ATM and recruited at break sites (Moyal et al., 2011). Depletion of RNF20/40 with siRNAs increased cells sensitivity to the radiomimetic drug Neocarzinostatin (NCS). Moreover it induced a delayed DDR shown by persistent γH2AX and 53BP1 foci without affecting the early steps of DDR activation

(Moyal et al., 2011). Monoubiquitination of H2B in response to DNA damage was found necessary to the recruitment of NHEJ factors XRCC4 and Ku80, and also of HR factors RPA, RAD51 and BRCA2 (Moyal et al., 2011) showing an involvement of RNF20/40-ATM signalling to play a role in both pathways. The role of H2Bub1 in HR was further investigated by another group, showing that resection is decreased in cells lacking RNF20 and that this depends on the damage-dependent interaction between RNF20 and NBS1 member of the MRN complex; indeed NBS1 mutants lacking the RNF20-interaction domain showed decreased resection, although NBS1-RNF20 interaction is not needed for each others' recruitment at DSBs (Nakamura et al., 2011). As previously mentioned, H2Bub1 histone mark is connected to H3K4me and H3K79me, and in yeast this connection is important for cell cycle arrest upon genotoxic stress, especially for H3K4me (Giannattasio et al., 2005). In mammals it was shown that H3K4me2 does not accumulate at laser-induced damage tracks thus suggesting that the role of H2Bub1 in DDR is independent on methylation of H3K4 (Moyal et al., 2011). On the other hand, ChIP experiments showed an RNF20-dependent increase of H3K4me at IsceI induced DSB (Nakamura et al., 2011). How H2Bub1 helps repair is still a matter of debate; one of the simplest explanation provided is that it might help recruitment of DDR factors through chromatin relaxation, in fact forced chromatin relaxation was shown to rescue the RNF20-depletion defect (Nakamura et al., 2011). Given the complexity of the phenotypes observed, it cannot be excluded that there are other mechanisms involved; for example ubiquitin on H2B might induce steric hindrance with other modifications that would play with recruitment and release of DDR factors, although it seems not to have an effect on 53BP1 recruitment (Wilson et al., 2016).

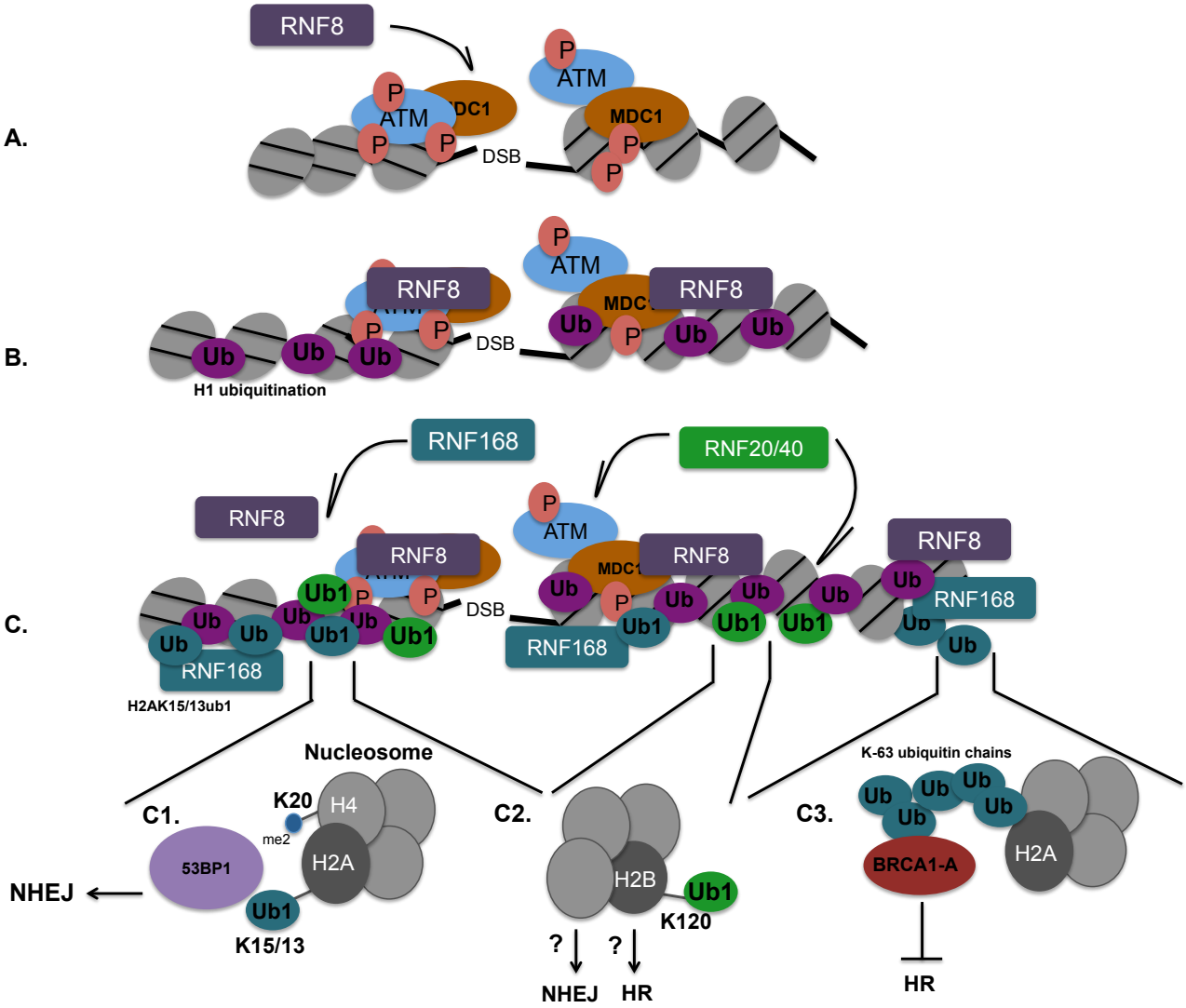

Figure 8 Ubiquitin signalling cascade at damage sites

(A) RNF8 is recruited via phosphorylated MDC1, and it catalyses the ubiquitination of linker histone H1. RNF168 is recruited in a RNF8-dependent manner. (C1) RNF168 catalyses the addition of monoubiquitin on K15 or K13 of histone H2A (H2AK15/13ub). H2AK15/13ub1 is recognized by 53BP1, that recognizes at the same time H4K20me2. (C3) RNF168 is also capable of extending, in an RNF8-dependent manner, K63-ubiquitin chains at the sites of break, which are needed for the subsequent recruitment of the BRCA1-A complex, through RAP80. The BRCA1-A complex is known to inhibit resection and thus HR. (C2) RNF20/40 dimer is recruited at the sites of breaks in response to irradiation, in an ATM-dependent manner, to induce monubiquitination of Histone H2B (H2Bub1) that has been shown to be required for both NHEJ and HR, and for the successful initial DDR signalling, although the exact mechanisms are still largely unknown.

P = phosphorylation; Ub = K63-linked ubiquitin chains; Ub1 = monoubiquitination

Deubiquitination of histone proteins involved in DDR

Consistently with the fact that ubiquitin-conjugating enzymes play an important role in regulating the DDR increasing evidences have shown that also DUBs, involved in the removal of ubiquitin, have a critical role. Considering the diversity of ubiquitin chains is not surprising that there are different kinds of DUBs showing some specificity for one substrate

or another. There are approximately 95 DUBs encoded by the human genome that fall in 5 distinct categories: Ubiquitin C-terminal Hydrolases (UCHs), Ubiquitin-Specific Proteases (USPs), Machado-Joseph Domain containing proteins (MJDs), Otubain domain-containing proteases (OTUs) and JAB1/MPN/Mov34 (JAMM) proteases. DUBs can regulate different processes in the cells, for my thesis I will focus on the role of DUBs in regulating DSBs repair (Figure 9).

K63-linked ubiquitin chains specific DUBs

As previously mentioned the formation of K63-linked ubiquitin chain at the site of break by RNF8/RNF168 is a key step of DSBs repair regulating the recruitment of the BRCA1-A complex, that contrarily to the canonical role of BRCA1 in promoting HR, inhibits resection. The BRCA1-A complex contains the DUB BRCC36. BRCC36 is a K63-linkage-specific DUB part of the JAMM proteases (Cooper et al., 2009) and it has been shown to localize at IRIF in a manner dependent on RAP80 (Sobhian et al., 2007). Consistently with the role of BRCA1-A complex, it was shown that targeting of BRCC36 to the damage counteracts the formation of excessive K63-ubiquitin chains, and that depletion of BRCC36 induced increased end resection with RAD51 loading, HR repair and sensitivity to irradiation, suggesting that excessive K63-ubiquitin chains lead to improper repair (Coleman and Greenberg, 2011; Hu et al., 2011). It is worth to mention that although depletion of BRCC36 increases HR it still stimulates a hyper-recruitment of 53BP1 to K63-linked ubiquitin chain, suggesting that 53BP1 stabilization to K63-linked ubiquitin chains is not sufficient to suppress HR.

However contrasting results have been obtained in the context of K63-ubiquitin chains specific DUBs. For example, KD of the Pad One Homolog (POH1) DUB also stimulates enlargement of 53BP1 foci at the breaks, without increasing NHEJ (Butler et al., 2012), but decreasing end resection, shown by a decrease in the formation of RPA foci (Kakarougkas et al., 2013). POH1 might cooperate with BRCA1 to remove 53BP1 from chromatin to promote HR, although is not clear yet how POH1 is recruited to chromatin. Moreover, depletion of OTUB2 (another K63-linkage specifc DUB part of the OTU DUB family) decreases HR, phenotype that is then rescued by concomitant depletion of 53BP1 (Kato et al., 2014). Thus it is confusing that depletion of BRCA1-A complex-component BRCC36 stimulates end resection, while depletion of OTUB2 has the opposite effects, being both DUBs that antagonize the stabilization of K63-ubiquitin chains. These contrasting results suggest that the complexity of these H2A ubiquitin chains and their role in recruiting downstream DDR factors is not fully understood.

Monoubiquitinated H2A specific DUBs

Other than polyubiquitin chains, also monoubiquitination of H2A, H2AX and H2B are involved in the DDR, and recent findings have broadened the role of DUBs targeting H2A.

USP3 is a homolog of yeast Ubp8 (known to deubiquitinate H2Bub1 as part of the DUB module of the SAGA complex). USP3 was shown to be a specific deubiquitinase for H2AK13/15ub counteracting the action of RNF168, and cells from mice deficient of USP3 shown increased formation of 53BP1 and γH2AX (Sharma et al., 2014). Overexpression screen using as a readout 53BP1 foci after irradiation found USP44 as another DUB involved in the counteracting of RNF168 induced ubiquitination of H2A, however is not clear if the target is H2AK119ub or H2AK13/15ub (Mosbech et al., 2013). It is clear that USP3 and USP44 might have redundant functions in cleaving ubiquitin ad DSB site; or they might be engaged at different chromatin territories and cell cycle stages (Chapman et al., 2012; Kakarougkas et al., 2013).

A similar overexpression study found USP26 and USP37 as regulator of HR through the recruitment of BRCA1-A complex. In fact depletion of USP26 and USP37 induced a decrease in HR that could be rescued by concomitant depletion of RAP80 (Typas et al., 2015). Again this study demonstrated how the RNF8/RNF168 ubiquitination cascade is probably involved in the balance between the HR and NHEJ regulating the binding balance between the HR-repressing and HR stimulating factors.

In an shRNA screen for chromatin remodelling factors it was discovered that depletion of USP51 increased 53BP1 and BRCA1 foci at damaged chromatin, functioning downstream of γH2AX formation and MDC1 recruitment, it was shown to be involved in the specific deubiquitination of H2AK13/15ub at damage sites. Moreover both depletion and overexpression of USP51 result in increased sensitivity to IR showing that the levels of USP51 must be tightly regulated for the proper repair (Wang et al., 2016).

H2Bub1 specific DUBs

While many studies have uncovered the role of H2A-specific DUBs in the regulation of the DNA damage response, little is known about the role of the H2B specific DUBs in DNA repair. In mammalian cells it has been shown only recently the role of RNF20 and RNF40 in the DDR via mono-ubiquitination of H2B (Moyal et al., 2011; Nakamura et al., 2011) but still little is known about the regulation of the deubiquitination of H2Bub1. The enzyme involved in the removal of mono-ubiquitin from H2B is USP22, part of the DUB module of the SAGA complex (described hereafter), together with structural proteins ATNX7, ATXN7L3 and ENY2. USP22 was found in a loss-of-function screen for factors involved in

CSR. CSR was used as a model to reveal new factors involved in the process and likely to be involved also in more general DSB repair. A mouse cell line was used in which successful CSR, from IgM to IgA, could be assessed by surface IgA expression after antigen stimulation (Ramachandran et al., 2016). In this way ENY2 depletion was found to inhibit the CSR from IgM to IgA, and further investigations showed that the same phenotype was observed in USP22 knock-out (KO) mice and ATXN7 depletion by shRNA (Ramachandran et al., 2016). These effects were shown to be independent on the function of the main enzyme involved in the process of CSR, Activation Induced cytidine Deaminase (AID) function (Ramachandran et al., 2016). It was shown that USP22-KO clones and ENY2-depleted mouse cells had a higher global level of H2Bub1, and a higher level of H2Bub1 at the Ig site, consistent with the role of the DUB module, after ionizing radiation and this was correlated with the defective CSR at the Ig locus. Although previous reports have shown that H2B monoubiquitination in response to ionizing radiation mainly depends on ATM (Moyal et al., 2011) here the authors show that ENY2 depletion and USP22 KO induced a defect in γH2AX formation that is dependent on both ATM and DNA-PK, thus reducing the CSR in mouse CH12 cells (Ramachandran et al., 2016). Previous reports showed that KD of RNF20/40 induced a decrease in HR efficiency and resection (Moyal et al., 2011; Nakamura et al., 2011) induced by reduction in H2Bub1 levels, thus one would expect that KD of the enzyme responsible for its deubiquitination would have opposite effects. However in mouse CH12 cells ENY2 and USP22 depletion induced as well a reduction in the efficiency of HR, NHEJ and Alternative-End joining pathways, showed by the DR-GFP assay (Ramachandran et al., 2016). Contrasting results might be explained by the different model systems (human vs mouse) and the specific cell type used in the latter, B cells.

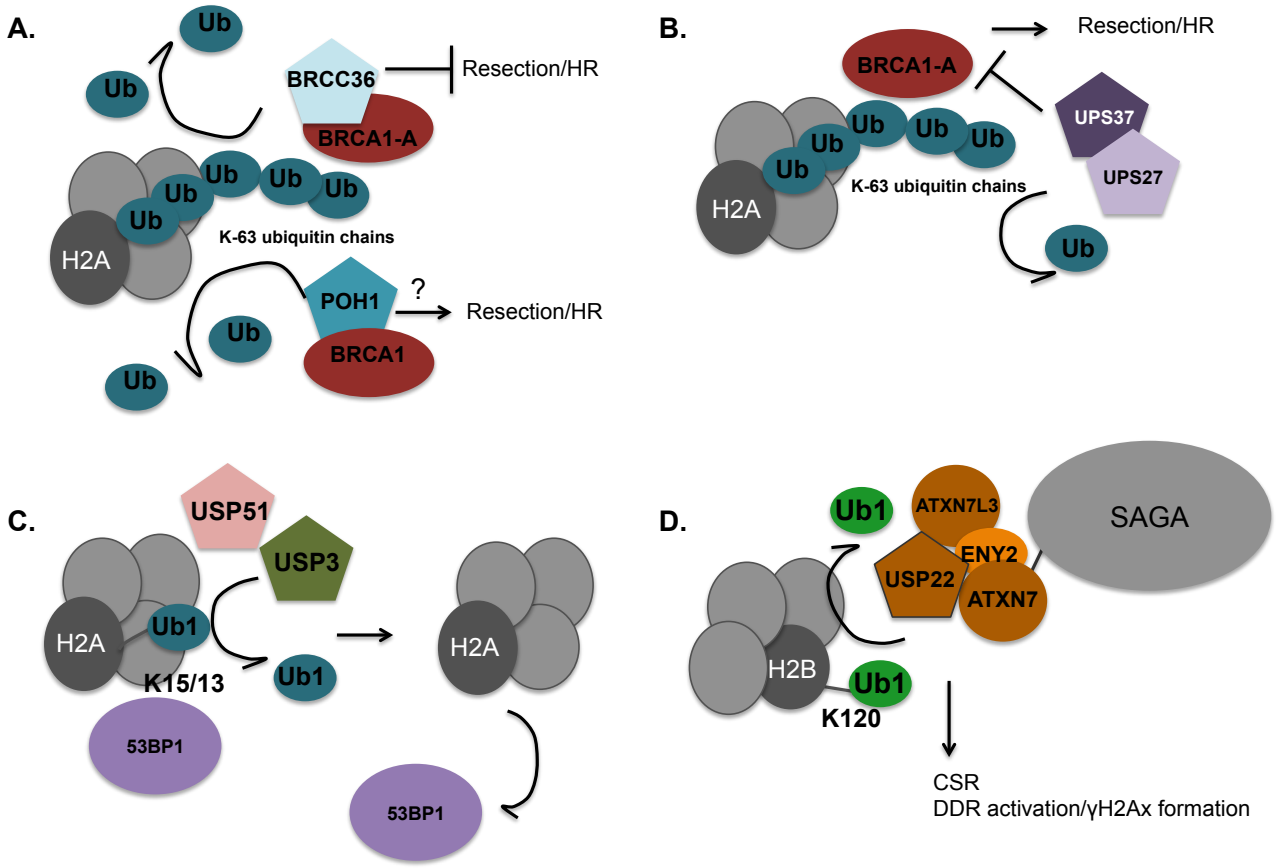

Figure 9 Role of DUBs in DNA repair

(A) Cleavage of K63-ubiquitin chains by BRCC36, part of the BRCA1-A complex counteracts resection and thus HR. POH1 deubiquitinase also counteracts K63-ubiquitin chains leading to the opposite effect. (B) BRCA1-A complex is recruited through RAP80 binding to K63-linked ubiquitin chains; USP37 and USP27 counteract BRCA1-A binding thus increasing resection and HR. (C) Removal of monoubiquitin from H2AK15/13ub catalysed by USP51 and USP3 counteracts 53BP1 binding to chromatin at damaged sites. (D) The removal of H2Bub1 catalysed by USP22, is needed for CSR and for the correct activation of the DDR in mouse B cells.

1.4 Nuclear compartments and their involvement in the repair process

The successful completion of the repair is driven by the efficiency of all the factors involved and by the efficient signalling, that relies on different post-translation modifications (PTMs) on histone and non-histone proteins. It is not surprising that histones modifications play a key role in DNA repair if we consider that the repair has to be completed in the context of chromatin, but also in the highly compartmentalized environment of the nucleus. Recent studies have shown that the efficiency of the repair and the choice of the pathway are made also accordingly to the chromatin environment and to the spatial localization of the damaged DNA. Several studies in yeast have shown that DSBs can relocate to different sites from where they have been generated for efficient repair.

1.4.1 Chromatin environments and nuclear compartments

To understand the mechanisms of breaks relocation it has to be understood that the linear molecule of DNA is carefully organized and arranged into the nucleus. First of all the double helix of DNA is wrapped around the nucleosome core composed of Histones H2A, H2B, H3 and H4, linked together by Histone H1 and forming the 10nm fiber so-called “beads on a string” (Luger et al., 1997a; Luger et al., 1997b). This structure is further folded into compacted 30 nm fibers (Luger et al., 2012). Accordingly to the cell cycle stage other level of compaction can arise, to reach, at the highest level, the structure of the metaphase chromosome. In addition, accordingly to specific histones and DNA modifications that give a high or low degree of compaction, two major components of chromatin can be differentiated; Heterochromatin and Euchromatin. It was almost 90 years ago when the two different states of chromatin were visualized (reviewed in: (Passarge, 1979)). Heterochromatin constitutes the most condensed chromatin, subdivided depending on the degree of compaction in constitutive and facultative heterochromatin. Constitutive heterochromatin harbours genomic regions that are repressed throughout the cell cycle and during all developmental or environmental changes; it is usually present in centromeres, repetitive regions and telomeres. On the contrary facultative heterochromatin characterizes region that might be silenced or activated in response to stimuli or developmental changes; as for example the inactivation of one female X chromosome. What is mostly used to characterized these different genomic regions relies on the presence or absence of specific and combinatorial histones PTMs; such as the trimethylation/dimethylation on K9 of Histone H3 (H3K9me3/H3K9me2) and the presence of Heterochromatin Protein 1 (HP1) in constitutive heterochromatin or the trimethylation of K27 on histone H3 (H3K29me3) and polycomb proteins in facultative heterochromatin (reviewed in (Grewal and Jia, 2007)). Euchromatin constitutes instead the active chromatin, which harbours the majority of constitutively transcribed genes, or tissue-specific active genes. Euchromatin is generally characterized by a higher degree of histone acetylation (such as acetylation of K9 on histone H3; H3K9ac) and the specific trimethylation of K4 of histone H3 (H3K4me3).

In addition, it is well known that heterochromatin and euchromatin are not randomly distributed inside the nucleus (Watson, 1955). Indeed electron microscopy images have shown that heterochromatin, which appears as a dense black structure, is localized in spots in the inner nucleus and as a ring around the nuclear periphery, while light euchromatic regions characterize the majority of the nucleoplasmic chromatin and

discontinuously interrupt the heterochromatic ring at the level of the nuclear pores, (the channels that allow the communication between nuclear and cytoplasmic compartments).

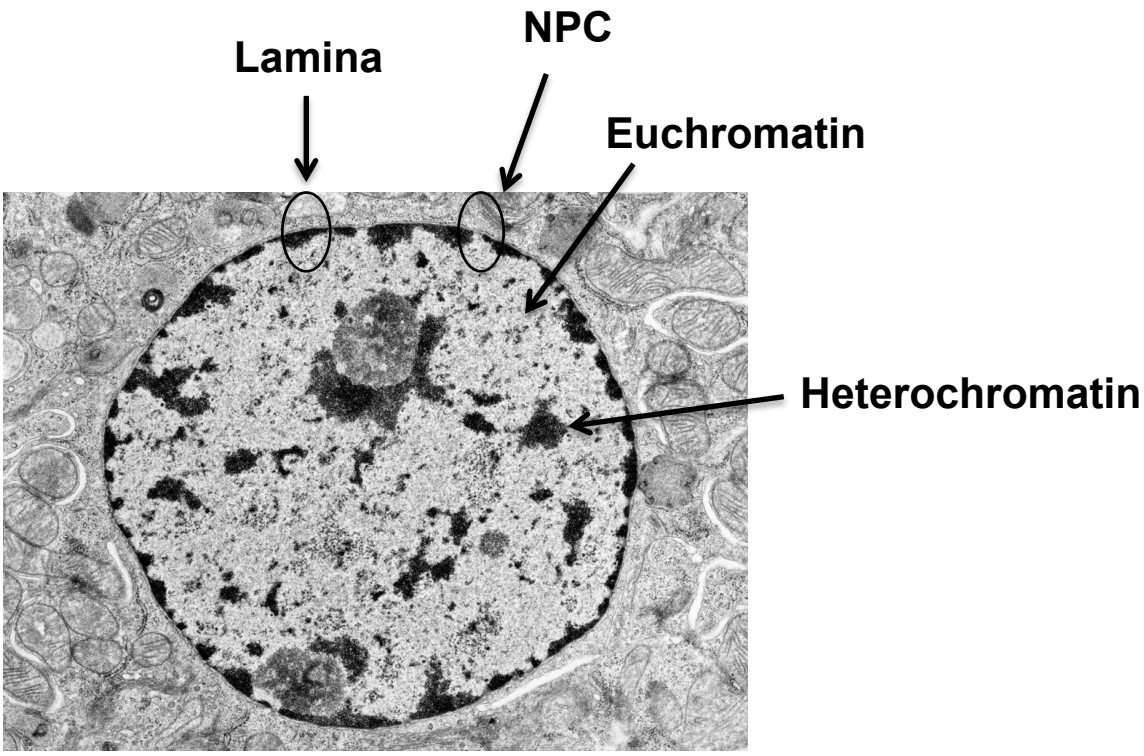

Figure 10 Electron microscopy image of a cell nucleus

Dense black regions show heterochromatic domains, while white regions show euchromatin. Heterochromatin represents a minor part of chromatin, and it interacts with the nuclear envelope; this interaction is interrupted at the level of the nuclear pores, which are characterized by euchromatic environment.
http://medcell.med.yale.edu/histology/cell_lab/euchromatin_and_heterochromatin.php

Thus, considering the above-described nuclear organization it is intuitive that the repair pathway and outcome varies according to the damage location, and it is not surprising that cells have developed mechanism to allow the damage to be repaired in the optimal conditions. Generally it is believed that the highly compacted structure of heterochromatin constitutes a barrier for repair factors and the repair machinery; that would prefer a more open structure to perform their action. Early reports showed that a DSB within the repetitive sequences of ribosomal DNA (rDNA), is relocated outside the nucleolus for efficient repair, which utilizes homologous recombination (Torres-Rosell et al., 2007). Failure do to so induced hyper-recombination in the rDNA locus with the subsequent excision of rDNA circles (Torres-Rosell et al., 2007). More recently it was shown both in *Drosophila* and mammalian cells that some breaks in heterochromatic regions move outside of the heterochromatic region to be properly repaired (Chiolo et al., 2011; Jakob et

al., 2011; Ryu et al., 2015; Tsouroula et al., 2016). Thus it is clear the chromatin environment and the chromatin compartment play a key role in the repair process.

1.4.2 Chromatin organization at the nuclear periphery

As mentioned before the Nuclear Envelope (NE) is characterized by different chromatin environments depending on the presence of nuclear pores. These structures perforate the NE and are mainly dedicated to the transport of mRNA and proteins in and out of the nucleus. In mammalian cells there are around 2000 NPCs per nucleus, and they are constituted by multiple copies of 30 different proteins called nucleoporins (nups) (Hoelz et al., 2011). Three different regions can be recognized in the NPC; the cytoplasmic side comprised of the outer ring and the spokes, a central channel comprised of inner ring nups and transmembrane nups, which create a channel in the nuclear envelope connecting the nucleus and the cytoplasm, and the nucleus side, composed of inner ring and a basket-like structure that connects the NPC with the inner nuclear environment (Figure 11) (Gay and Foiani, 2015).

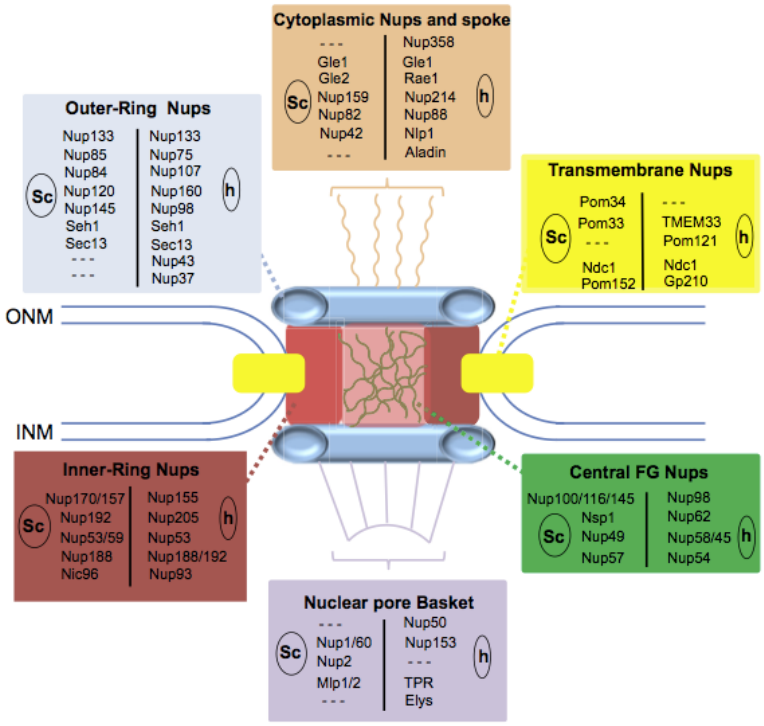

Figure 11 Schematic representation of the Nuclear Pore Complex (NPC)

Saccaromyces cerevisiae (Sc) and human (h) components of the NPC are depicted. ONM: outside nuclear membrane; INM, inner nuclear membrane. (Gay and Foiani, 2015)

NPCs perforate the NE, a lipo-proteic double membrane that physically separates nucleus and cytoplasm. On the nuclear side, the Inner Nuclear Membrane (INM) is covered by a network of proteins called Lamins. Genomic regions interacting with Lamina are called Lamina Associated Domains (LADs) and have been shown to have common features between *Drosophila*, mouse and human cells (Guelen et al., 2008; Kind and van Steensel, 2014; Peric-Hupkes et al., 2010; Peric-Hupkes and van Steensel, 2010; Pickersgill et al., 2006). LADs harbour generally gene-poor regions, low levels of gene expression and are characterized by high levels of H3K9me2, but no H3K4me3 or H3K79me2.

1.4.3 Role of nuclear envelope in genome stability

A plethora of studies in yeast have made clear the importance of proteins part of the nuclear envelope in the repair process. Nups deletion mutants, such as nup133Δ , nup84Δ and nup120Δ, have been shown to be more sensitive to DNA damaging agents, and accumulate spontaneous Rad52 foci (Loeillet et al., 2005) suggesting repair defects. Moreover these defects were shown to be dependent on the presence of the E3 sumo ligase Ulp1 at the NPC, tethered by the Nup84 complex and Nup60 together with Mlp1/Mlp2 (Palancade et al., 2007). The sumoylation signalling cascade, generated by Ulp1 with the sumoylation of yeast Ku80, was shown to be, at least in part, the cause of accumulation of unrepaired DNA (Palancade et al., 2007). The importance of the NPC in DNA repair in yeast is also confirmed by more recent studies showing the localization of damaged DNA at the nuclear pore. It was shown that a single DSB, induced with the HO restriction enzyme at the mating locus, was relocated at the nuclear periphery when encountering difficulties in finding the homologous sequence for repair (Nagai et al., 2008). This happened in haploid strains (Nagai et al., 2008), or in strains where the sequence was deleted or far away from the break (Oza et al., 2009), but did not happen in absence of damage or when the homologous sequence was located in the vicinity of the break (Nagai et al., 2008; Oza et al., 2009). These results suggested that a “difficult-to-repair” break might direct a relocation of the damaged chromatin to facilitate repair and to avoid deleterious outcomes, such as unscheduled recombination between sequences harbouring random homology. Indeed the process was shown to be dependent on the integral nuclear envelope protein Mps3 and the association with the periphery, through Mps3, seems to be a way to slow the rate of recombination in yeast cells (Oza et al., 2009). However, restriction enzyme-directed breaks were shown to be equally bound by nucleoporins in yeast, such as nups part of the Nup84 complex (Nagai et al., 2008), thus discrimination between nuclear envelope and NPC association has to be done given the

difference in chromatin composition; indeed break relocation to one or the other might have different meanings and outcomes. Nuclear periphery and NPC binding could be differentiated by high-resolution fluorescence microscopy in yeast (Horigome et al., 2011) and it was elucidated that in yeast cells DSBs association with the NPC happens in G1 and S phase, and is independent from the presence of recombination protein Rad51 (Horigome et al., 2014), while the association with the nuclear membrane protein Mps3 is restricted to S phase and dependent on Rad51 (Horigome et al., 2014; Kalocsay et al., 2009; Oza et al., 2009), thus confirming a different outcome in terms of repair. In conclusion, with the available data, it has been suggested that interaction with the nuclear periphery might have a repressive role for HR while the NPC might represent a more permissive environment (Horigome et al., 2014).

In accordance with this model elegant experiments in mammalian cells disclosed the different role of these two compartments in DNA repair, following the fate of a single DSB induced in an artificially-tethered locus at the lamina or at the pore. These experiments showed that a DSB induced at the lamina, successfully recruits NHEJ factors Ku80 and XRCC4 while the recruitment of HR factors BRCA1 and RAD51 is markedly reduced, demonstrating that the lamina is a repressing environment for homologous recombination, and that this is due to the repressive chromatin environment (Lemaitre et al., 2014). In fact inducing local or general chromatin decondensation is able to rescue the HR defective phenotype of the lamina-targeted DSB (Lemaitre et al., 2014). At the same time a DSB specifically induced at the NPC did not shown any HR defect, suggesting and confirming the NPC constitute a permissive environment for recombinational repair (Lemaitre et al., 2014). However, in mammalian cells, nor the single DSB induced with restriction enzyme, nor breaks induced globally with genotoxic agent NCS, showed any global rearrangement and mobility from one compartment to another (Lemaitre et al., 2014). This is in accordance with previous studies in mammalian cells, where it has been demonstrated that multiple DSBs on several chromosomes do not cluster nor move to the periphery to be repaired (Soutoglou et al., 2007). Although it has not been demonstrated in mammalian cells that damaged chromatin can travel relatively long distances and relocate at the NPC, several nucleoporins have been implicated in the repair process.

1.4.4 Role of Nucleoporins in DDR in mammalian cells

As mentioned above, studies in yeast and *Drosophila* have shown a relocation of DSBs to the NPC, while in mammals the situation appears to be more complicated. While it has been shown that breaks in heterochromatic region can relocate outside of highly

compacted chromatin for repair (Tsouroula et al., 2016) travelling for short distances, single DSBs in the inner nucleus seem not to relocate to the nuclear periphery, yet proteins that are part of the NPC have a conserved role in repair due probably also to their dynamic exchange between the NPC and the nucleoplasm. Early studies showed that the NUP153, part of the nuclear pore basket, plays a role in the choice between HR and NHEJ (Lemaitre et al., 2012). It was shown that human U2OS cells depleted of NUP153 are more sensitive to DNA damaging agents and they are defective in the G2/M checkpoint activation; the rate of NHEJ was significantly decreased in NUP153 KD while the rate of HR was increased (Lemaitre et al., 2012) (It must be mentioned that different reports showed opposite effects on HR, probably due to the different type of cell line used); this is counterintuitive if we think that NPC are generally considered HR-prone regions, but this shows that nucleoporins can affect the DDR in multiple ways in mammalian cells. In fact depletion of NUP153 affects the nuclear import of 53BP1 (Lemaitre et al., 2012), and this can partially explain the effects on NHEJ and HR, being 53BP1 a resection-blocking factor. Moreover, it was later shown that NUP153, together with another basket nucleoporin NUP50, is not only required for 53BP1 nuclear import but also for its correct localization ad damage sites, indeed 53BP1 fused to a potent nuclear localisation signal (NLS) still showed defects in forming genome wide foci upon NUP153 KD, although correctly localized in the nucleus (Mackay et al., 2017). Although NUP153 and NUP50 are known to exchange between NPC and nucleoplasm they did not show damage localization (Lemaitre et al., 2012; Mackay et al., 2017) thus NUP153 has a role in targeting 53BP1 to damage sites, that is dependent on NUP50, without localizing to the damage itself.

The role of nucleoporins in DNA repair is only recently being investigated in mammalian cells with the realization that there is a not linear interplay of nups in the DDR. Different nups can play different non-redundant roles. Indeed it has been recently discovered a role in the DDR for a third basket nucleoporin, TPR, homolog of yeast Mlp1 and Mlp2. Depletion of TPR increases cell sensitivity to DNA damaging agent NCS, and it decreases both NHEJ and HR efficiency. At the same time, although it has been previously shown that depletion of NUP153 affects basket formation and TPR levels and localization at the basket (Hase and Cordes, 2003; Lelek et al., 2015), depletion of TPR did not affect the DDR at the same way of NUP153; indeed depletion of TPR did not affect nuclear import of 53BP1 nor its localization ad damage sites (Duheron et al., 2017). However depletion of TPR and NUP153 affects resolution of BRCA1 and 53BP1 foci at later time points after NCS treatment (Duheron et al., 2017). Among the different factors that affect 53BP1

binding to damage sites, it was shown that SUMOylation is required for efficient accumulation of 53BP1 (Galanty et al., 2009). A new piece of data was added with the discovery that NUP153 and TPR are required for the localization and stability of the SUMO protease SENP1 at the nuclear pore, and this was shown to be required for efficient 53BP1 sumoylation and targeting to damage. However NUP153 and TPR again seem to have different mode of actions, considering that forced tethering of SENP1 at the NPC could restore the NHEJ defect seen in NUP153 KD but not in TPR KD (Duheron et al., 2017). These data suggest that nups part of the nuclear pore basket are indeed, in different ways, important for a faithful DDR signalling and thus repair, however further studies will be necessary to asses the exact role of each of them and the mechanisms involved.

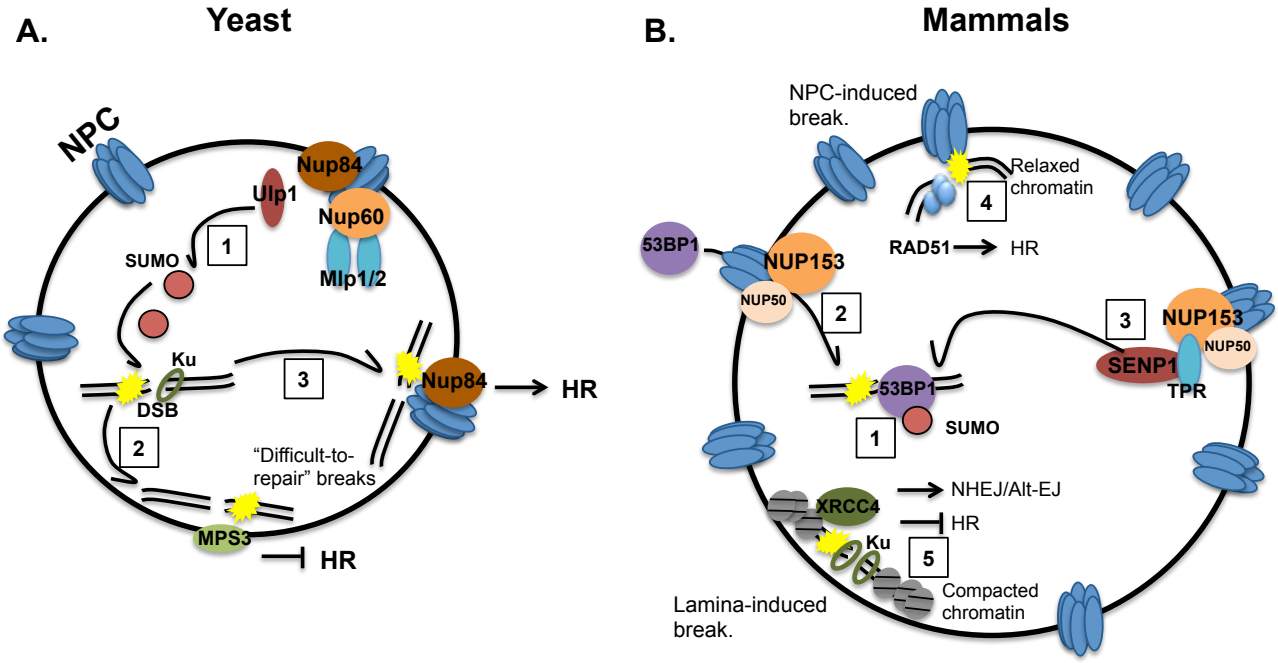

Figure 12 Involvement of the nuclear periphery in DNA repair

(A) In yeast nucleoporins protect the genome integrity through mechanisms involving Ulp1 E3 SUMO ligase. (1) Breaks induced in the nucleus that are difficult to repair, have been shown to relocate at the nuclear periphery. (2) Periphery relocation through Mps3 slows the recombination rate. (3) NPC relocation promotes HR. (B) (1) In mammalian cells breaks that are induced in the inner nucleus have not been shown to relocate at the nuclear periphery. However nucleoporins have been shown to have a conserved role in DNA repair. Basket nups, such as Nup153 and TPR are involved in 53BP1 targeting to damage sites, through (2) nuclear import and (3) through sumoylation. Nuclear environments at the NPC and at the lamina directs repair in a similar manner compared to yeast cells. (4) NPCs are a favourable environment for HR while (5) lamina associated domains rely mostly on NHEJ or Alt-EJ for repair.

1.5 NPC interacting complexes: SAGA and TREX-2

The NPC is anchored to the NE, thus some structural nucleoporins are stably associated within the NE, while others, such as the nups of the basket might diffuse into the nucleoplasm and interact with several other proteins, to accomplish basic functions such as mRNA and protein export, both in yeast and higher eukaryotes. In addition many adaptor proteins might mediate the interaction between the NPC and the inner nuclear environment and contribute to the additional roles of the nuclear pore in genome stability maintenance. Important examples of these adaptors in yeast and in mammals are the Spt-Ada-Gcn5-Acetyltransferase (SAGA) co-activator complex and the TRanscription and EXport complex 2 (TREX-2). Interestingly SAGA and TREX-2 share one subunit, ySus1/hENY2, a small protein of 11kDa with no enzymatic activity but fundamental for the mRNA export activity of TREX-2 and the deubiquitinase activity of the DUB module of SAGA. TREX-2 and SAGA define an interface of interactions that is necessary for the accomplishment of transcription, RNA biogenesis, mRNA export and protection from genome instability that will be described in the following section.

1.5.1 SAGA

The SAGA complex is a highly conserved 1.8 MDa transcriptional coactivator that is composed of 19 subunits in yeast and 20 in mammals (Table 1) (Helmlinger and Tora, 2017). Subunits of the SAGA complex are organized in distinct modules; these include a structural module, a TATA-binding protein (TBP) interacting module, a splicing module and two modules that exert enzymatic activities in the context of histones PTMs. Given the importance of chromatin modifications in several chromatin-associated events it is not surprising that the last two modules are important for the transcription function of the SAGA complex.

	Yeast SAGA	Human SAGA
HAT module	GCN5	GCN5/PCAF
	Ada2	ADA2B
	Ada3	ADA3
	Sgf29	SGF29
DUB module	Sus1	ENY2
	Sgf11	ATXN7L3
	Sgf73	ATXN7
	Ubp8	USP22
Core Structural module	Taf5	TAF5
	Taf6	TAF6L
	Taf9	TAF9/TAF9b
	Taf10	TAF10
	Taf12	TAF12
	Spt7	SUPT7L
	Ada1	TADA1
	Spt20	SUPT20H
TBP binding	Spt3	STP3
	Spt8	-
TF-binding	Tra1	TRAAP
Splicing-module	-	SF3B3
	-	SF3B5

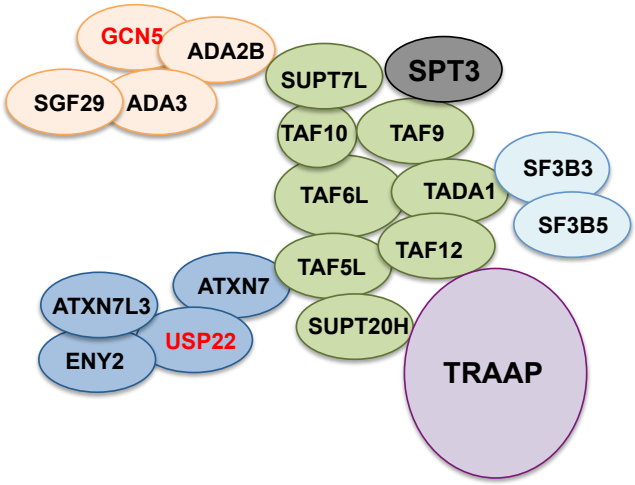

Table 2 Subunits of the SAGA complex in yeast and humans

In red are depicted the subunits harbouring enzymatic activity in the Histone Acetyltrasferase (HAT) module, catalysing the addition of an acetyl group on H3K9, and in the deubiquitination module, catalysing the removal of ubiquitin from mono-ubiquitinated H2B (H2Bub1). Adapted from (Helmlinger and Tora, 2017)

The HAT module in mammals contains four subunits; SGF29, ADA2b, ADA3 and the acetyltransferase enzyme GCN5 that is able to catalyse the addition of acetyl group on K9 and K14 of histone H3 (H3K9ac and H3K14ac) (Bonnet et al., 2014; Brand et al., 1999; Feller et al., 2015). Gcn5 was the first subunit of SAGA to be identified in yeast (Grant et al., 1997). It was shown that association of Gcn5 with a bigger complex was necessary for the *in vivo* HAT activity of the enzyme (Grant et al., 1997); given the presence of already discovered Ada and Spt proteins in the complex this latter was named Spt-Ada-Gcn5 Acetyltrasferase, hence SAGA, complex (Grant et al., 1997). The DUB module of the human SAGA contains four subunits as well, ATXN7, ATXN7L3, the adaptor ENY2 and

the enzyme USP22. These subunits were the last subunits to be discovered in yeast as part of the SAGA complex, required to remove ubiquitin from K123 (the equivalent of K120 in mammals) of monoubiquitinated H2B (Gavin et al., 2002; Henry et al., 2003; Sanders et al., 2002). The deubiquitination of H2Bub1 by the yeast homolog of USP22 (Ubp8) was found to be associated with transcriptional activation and Ubp8 was found to be recruited at promoters together with Gcn5 (Henry et al., 2003). Interestingly deletion of DUB's or HAT's subunits does not affect the integrity of the SAGA complex and does not alter each other's enzymatic activities (Henry et al., 2003; Lee et al., 2011). Human SAGA was also characterized in human HeLa cells, and it was found to be a transcription co-activator in chromatin-assembled promoter *in vitro* (Martinez et al., 2001). It is worth to mention that, for long time after SAGA characterization, it was believed that SAGA would be involved in the transcription of only a subset of genes, corresponding roughly to the 10% of yeast genome (Huisinga and Pugh, 2004; Lee et al., 2000; Tirosh and Barkai, 2008; Venters et al., 2011). These genes were characterized to be stress-response genes activated after environmental changes (Huisinga and Pugh, 2004; Lee et al., 2000). However discrepancies between genes whose mRNA levels were affected by SAGA depletion and genes' promoters bound by SAGA were observed, suggesting that probably ChIP of SAGA subunits, or analyses of steady state levels of mRNA could not reflect the real behaviour of the SAGA complex in live cells. In fact, more recent studies revealed the dynamicity of this complex, acting on all expressed genes and required for their transcription (Baptista et al., 2017; Bonnet et al., 2014). It was shown that the DUB module is required for the deubiquitination of global H2Bub1, along gene bodies of all transcribed genes while the HAT activity is restricted to promoters of all active genes (Bonnet et al., 2014). Interestingly a very recent paper suggested that the DUB module might work independently on the HAT module in *Drosophila*'s embryos, where it was shown that the two modules are required at different stage of development (Li et al., 2017).

1.5.2 TREX-2

The TREX-2 complex is a highly conserved complex among eukaryotes. In yeast and mammals is composed of five bona-fide subunits, number that can be slightly variable in other organisms (Table 3). In mammals it contains Germinal Center Associated Nuclear Protein (GANP), Enhancer of Yellow-2 (ENY2), PCI Domain Containing 2(PCID2), 26 proteasome subunit SEM1 (SEM1 or DSS1) and Centrin2 or Centrin3, that in yeast are respectively Sac3, Sus1, Thp1, Sem1 and Cdc31. In *Drosophila* a complex containing the *Drosophila* X-linked male sterile (Xmas)-2 (homolog of Sac3), E(y)2 (homolog of ENY2)

and PCID2 was purified and it was called AMEX to distinguish from the yeast and mammalian TREX-2 (Kopytova et al., 2016). Moreover in *Arabidopsis thaliana* three homologs of yeast Sac3 were characterized, SAC3A, SAC3B and SAC3BC, and among them, SAC3A and SAC3B were shown to be part of a complex containing THP1, DSS1 and Centrins. (Lu et al., 2010), whereas the *Arabidopsis* homolog of Sus1 was not found to interact with any of these subunits (Lu et al., 2010)

A.

Yeast (y) TREX-2	*Arabidopsis* TREX-2	*Drosophila* (d) AMEX	Human (h) TREX-2
Sac3	Sac3B-SAC3A	Xmas-2	GANP
Sus1 (x2)	-	E(y)2	ENY2 (x2)
cdc31	Centrin1-Centrin2	-	Centrin2 – Centrin3
Thp1	THP1	PCID2	PCID2
Sem1	DSS1	-	DSS1

B.

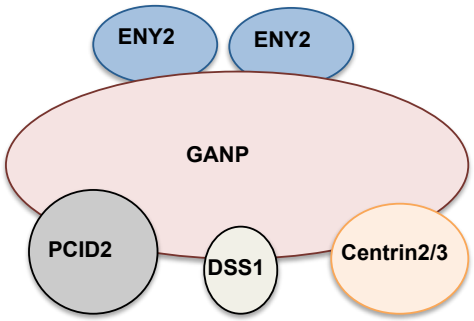

Table 3 Subunits of the TREX-2 complex among the different organisms

(A) Different composition of the TREX-2 complex among organisms. (B) Schematic representation of the mammalian TREX-2 complex, containing the scaffold subunit GANP, two ENY2 subunits, PCID2, DSS1 and Centrin2 or Centrin3.

The widely accepted role that has been assigned to the whole TREX-2 complex, in yeast mammals and *Drosophila*, is mRNA export; indeed depletion of each subunit of the complex induces accumulation of mRNA in the nucleus. However it is worth to mention that in *Arabidopsis*, depletion of THP1 causes export defects while none of the SAC3 homologs seems to be involved in mRNA export (Lu et al., 2010). The partial structure of the complex was resolved in yeast and mammals and it revealed a strong conservation, however little differences in the structure and in the process of export from yeast to mammals probably contributed to the diversification of the some TREX-2 functions.

TREX-2 crystal structure

The crystal structure of the yeast Sac3:Sus1:Cdc31 complex was resolved by crystallography in 2009 and it was shown Sac3 Cdc31 Interacting Domain (CID) region binds two Sus1 chains (Sus1A and Sus1B) and one Cdc31 chain (Jani et al., 2009). The CID domain forms an extended α-helix, on which two Sus1 subunits, constituted by 5 α-

helices joint by flexible hinges, are wrapped around (Jani et al., 2009). It is worth mentioning that extended α-helices are unstable, thus probably one Sus1 subunit would not be enough to cover the long Sac3 α-helix and to stabilize it (Jani et al., 2009) (Figure 13). The crystal structure of the human GANP CID region showed that, also in human cells, two copies of ENY2 are wrapped around the long CID α-helix but with a different orientation compared to the yeast structure (Jani et al., 2012) that results in a different exposition of GANP α-helix compared to Sac3 and might account for different interactions (Figure 13). As GANP, also ENY2 it was shown to be an unstable protein in the absence of his partner (Umlauf et al., 2013).

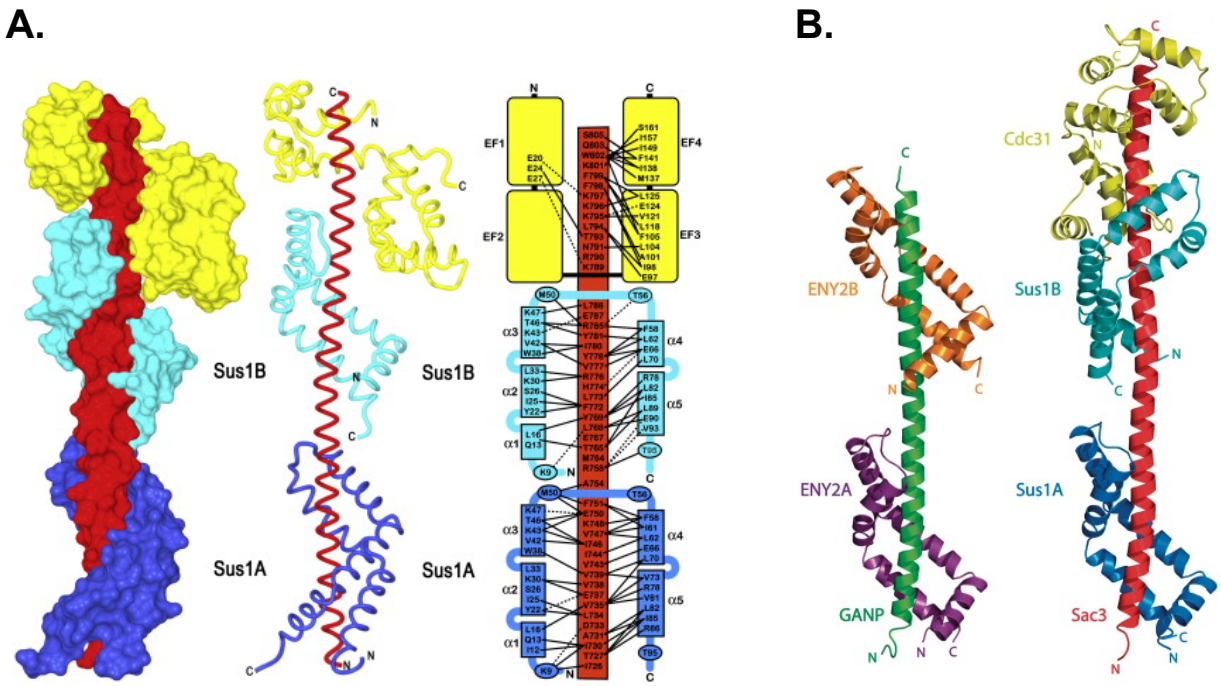

Figure 13 Crystal structure of the Sac3:Sus1:Cdc31 in yeast

(A) In red is depicted the long α-helix of the Sac3 CID domain. In dark blue and light blue are depicted the two Sus1 subunits, wrapped around Sac3. In yellow is depicted the Cdc31 subunit of the yeast complex (Jani et al., 2009). (B) Crystal structure of the human GANP CID domain associated with the two ENY2 subunits in orange and purple. On the left is depicted the yeast crystal structure; that reveals the difference between the orientation of the two Sus1 and the two ENY2 subunits (Jani et al., 2012).

A structure of the total TREX-2 complex has been difficult to obtain, given the natural low levels of Sac3 and to the fact that overexpression of Sac3 is lethal in yeast cells due to sequestration of Cdc31 from its other partners and the instability of the Sac3 crystals in their C-termini (Aibara et al., 2016; Fischer et al., 2002; Jani et al., 2009). However other

than the Sac3:Sus1:Cdc31 submodule also the structure of the Sac3 region binding to Thp1 and Sem1 was recently obtained (Aibara et al., 2016).

TREX-2 characterization

The first subunit of the TREX-2 complex to be discovered was the scaffold protein Sac3 in yeast cells. A study from 1996 characterized the novel yeast gene *SAC3*, being involved in actin polymerization and in cell cycle progression (Bauer and Kolling, 1996; Jones et al., 2000). Successively Sac3 was shown to be physically part of the NPC, interacting with Nup1 and Nup60, the yeast homologs of mammalian NUP153, and it was found to be important for protein export, (Jones et al., 2000) but later on, its main role in mRNA export was discovered. Indeed it was shown that Sac3 depletion, and surprisingly also overexpression, would cause severe mRNA export defects, but not tRNA or ribosomal RNA (Fischer et al., 2002). Moreover, the mRNA export activity was found to be dependent on Sac3 localization at the inner side of the nuclear pore by its C-terminal domain in yeast. Indeed Sac3 mutants lacking the C-terminal domain showed growth and mRNA export defects that were complemented by the fusion of the truncated Sac3 with Nup60 (Fischer et al., 2002).

In the same study it was characterized for the first time a complex containing Sac3 and Thp1, a protein already shown to be involved in transcription elongation and transcription dependent recombination (Gallardo and Aguilera, 2001). The novel Sac3-Thp1 was found to be associated with the nuclear pore and the interaction being dependent on Sac3 presence, indeed in Sac3Δ strains Thp1 no longer showed nuclear envelope pattern (Fischer et al., 2002). This was the first study that characterized the first two components of the TREX-2 complex, Sac3 and Thp1 (respectively GANP and PCID2 in mammalian cells), as integral members of the mRNA export machinery. The other three subunits of the complex, Sus1 (hENY2), Cdc31 (hCENTRIN2/3) and Sem1 (hDSS1) were discovered few years later in this order. Sus1 was already discovered (described in details hereafter) when yeast centrin Cdc31 came into play as a member of the complex. The best-characterised role for centrins is the cell cycle-dependent duplication of microtubule organizing centers, thus it was surprising to find a new role in mRNA export for Cdc31 as integral member of the Sac3-Thp1-Sus1 complex. Cdc31 was shown to interact directly with Sac3, independently on Sus1 or Thp1 (Fischer et al., 2004) and the interaction domain, between aminoacid 733 and aminoacid 860 was called CID for Cdc31 Interacting Domain (Fischer et al., 2004). Cdc31 was found to associate also with the nuclear pore,

other than with the spindle pole body, and the suppression of Cdc31 expression induced mRNA export defects, but not tRNA or ribosomal subunits (Fischer et al., 2004).
The Sac3-Thp1-Sus1-Cdc31 new complex, involved in mRNA export was hence called TREX-2, following the nomenclature of an already existing complex involved in mRNA export called TREX (Kohler and Hurt, 2007).
Successively the last subunit of the complex was discovered, again in yeast. Sem1 is a regulatory particle of the proteasome, hence again it was surprising to find its association with TREX-2 and involvement in mRNA export. Sem1 depletion induced mRNA export defects comparable to TREX-2 mutants and Sem1 co-purified with Sac3 and Thp1 (Faza et al., 2009). In addition Sem1 depletion was shown to have the same transcription-associated recombination of previously reported Sac3Δ and Thp1Δ strains (Faza et al., 2009; Gonzalez-Aguilera et al., 2008).
The discovery of Sus1 in yeast is of particular importance given its association with the co-activator SAGA suggesting a connection between the two complexes (Rodriguez-Navarro et al., 2004). Interestingly, this dual association is conserved in *Drosophila* and in mammals (Georgiev, 1994; Georgieva et al., 2001; Jani et al., 2012; Zhao et al., 2008). However, while in yeast it has been shown that SAGA and TREX-2 are physically connected (Kohler et al., 2008; Rodriguez-Navarro et al., 2004) in mammals MS data showed that ENY2 co-immunoprecipitates with SAGA and TREX-2, however no other TREX-2 subunit is part of SAGA and *vice versa* (Umlauf et al., 2013).

1.5.3 Sus1 discovery and SAGA-TREX-2 interplay

The Sac3-Thp1 complex was already documented when a novel associated protein was found, Sus1 (ENY2 in mammals) (Rodriguez-Navarro et al., 2004). The gene of *SUS1* was found in a synthetic lethal (sl) screen for genes that would be lethal in combination with mRNA export factor Yra1. The gene was mapped on yeast chromosome 2, and was found to code for a 96 aminoacids protein, named Sus1 (for Sl gene Upstream of *YSA1*). Interestingly Sus1 was found to co-purify not only with the Sac3-Thp1 complex, but also with the proteins of the previously characterized SAGA complex, involved in transcription. Furthermore is was shown that Thp1 co-purified with the bona fide SAGA component Ada2, however depletion of Ada2 and other SAGA subunits did not induce a nuclear accumulation of mRNAs thus suggesting that although the two complexes interact, SAGA is not involved in mRNA export (Rodriguez-Navarro et al., 2004). In addition, at least a part of Sus1 population was found to be located at the nuclear pore, and this localization being Sac3 dependent, in the same way as Thp1 (Rodriguez-Navarro et al., 2004). ChIP

experiments also showed Sus1 binding at the promoter of the galactose-induced GAL1 gene, suggesting that Sus1 might be involved in both transcription and export to recruit mRNA export factors to chromatin or, in another view, to tether activated genes to the nuclear pore in the process so-called “gene gating”. The gene gating hypothesis was proposed in 1985 by Blobel, and suggests that transcribed chromatin would relocate close the NPC to facilitate the export of the mRNA molecule from the nucleus to the cytoplasm (Blobel, 1985). This was shown to be true in yeast, at least for some inducible genes, whose transcription will be activated in response to environmental stimuli, such as GAL genes (Casolari et al., 2004). The repositioning of active GAL genes to the nuclear pore is decreased in Sac3Δ, Sus1Δ and Thp1Δ strains (Cabal et al., 2006; Chekanova et al., 2008) confirming a role for the whole TREX-2 complex in tethering active genes to the NPC. Although not all SAGA mutants showed the same reposition defect (Cabal et al., 2006; Chekanova et al., 2008), the connection between transcription and export in yeast is at the bases of SAGA-TREX-2 interplay. The discovery of Sus1 and its recruitment to promoters of transcribed genes in a TREX-2 and SAGA-dependent manner suggests that TREX-2 and SAGA work synergistically to recruit Sus1 to chromatin to promote elongation and subsequent export (Figure 14) (Garcia-Oliver et al., 2012; Pascual-Garcia et al., 2008; Rodriguez-Navarro et al., 2004).

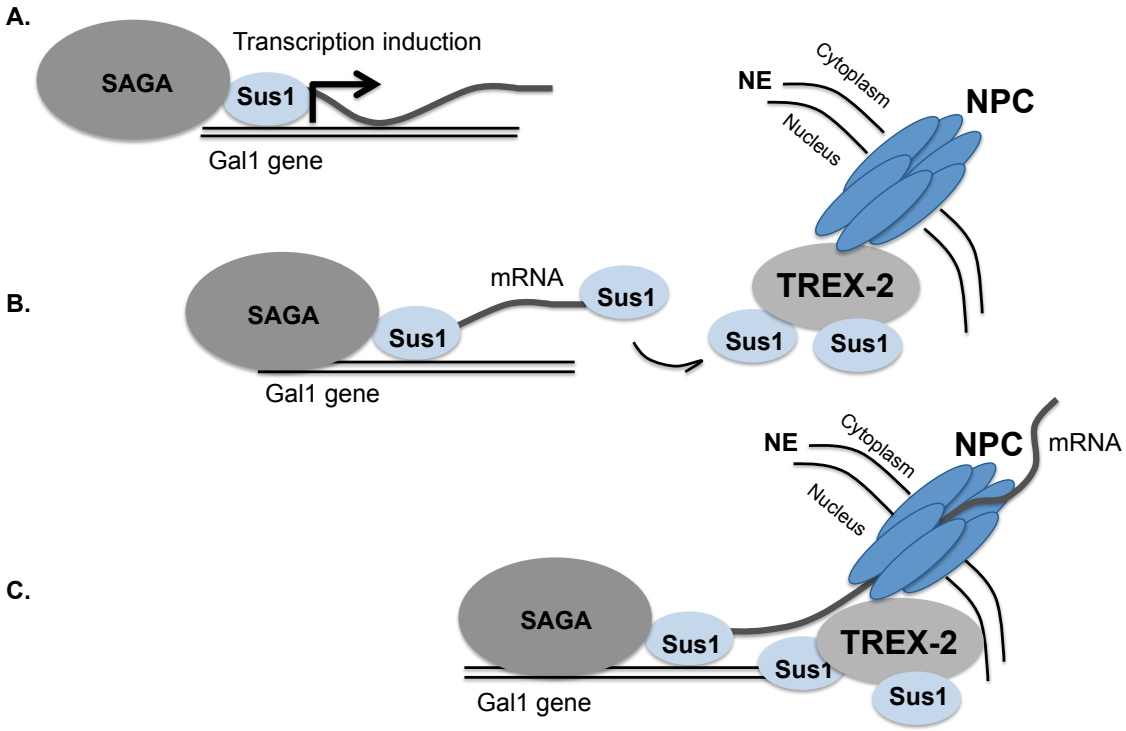

Figure 14 Schematic overview of the gene gating mechanism in yeast

The gene gating in yeast is regulated by the coordinated action of SAGA and TREX-2 through the common subunit Sus1. (A) Galactose induction stimulates the transcription of the *GAL1* gene. Sus1 is recruited at the GAL1 promoter in a SAGA-dependent manner and is required for Gal1 transcription. (B) Sus1 is needed for transcription elongation of the Gal1 mRNA and for the tethering of the activated gene to the NPC for export. (C) Subunits of the TREX-2 complex are involved in the relocation of the transcribed chromatin to the NPC and for the subsequent export of the mRNA; the process is called gene-gating. NE = Nuclear Envelope; NPC = Nuclear Pore Complex

1.5.4. TREX-2, SAGA and genomic instability in yeast

The gene gating mechanism described in yeast and its dependence on SAGA-TREX-2 interaction was proposed to be involved in protecting genome stability. As already mentioned, *THP1* gene was discovered in a screen for yeast deletion mutants influencing the recombination (Gallardo and Aguilera, 2001). A plasmid-based assay allowed measuring the frequency of recombination between two repeated sequences that would eventually form a functional gene only after recombination (i.e. *LEU+* gene) (Prado and Aguilera, 1995; Prado et al., 1997). Deletion of Thp1 induced an increase in recombination of more than 130 fold above the WT levels, hence called hyper-recombination phenotype. It was shown that this hyper-recombination is a consequence of transcription elongation in the intervening sequence between the repeats (Gallardo and Aguilera, 2001). If transcription is abolished, or the intervening sequence is deleted, the hyper-recombination

phenotype is rescued (Gallardo and Aguilera, 2001). Given the strong physical association between Sac3 and Thp1, similar analyses were conducted in Sac3Δ strains that displayed the same identical hyper-recombination phenotype of Thp1Δ strains suggesting that Sac3 and Thp1 form a complex that acts as a functional unit (Gallardo et al., 2003). Moreover, the same phenotype was observed in Sus1Δ strains confirming a role, for the whole TREX-2 complex in protecting the yeast genome from genomic instability (Gonzalez-Aguilera et al., 2008).

This hyper-recombination phenotype was observed in other mutants of the mRNA export machinery, and some nucleoporins (such as Nup60), but it was not observed in transcription mutants (Gonzalez-Aguilera et al., 2008; Rondon et al., 2003). Given the association of Sus1 with the SAGA complex, also SAGA mutants were analysed using the same direct repeat system, but no hyper-recombination was observed in none of the mutants, suggesting that Sus1 shares function with the TREX-2 complex in the maintenance of genome integrity (Gonzalez-Aguilera et al., 2008). Analyses on Cdc31 were instead more challenging, being Cdc31 an essential gene in yeast and thus only certain mutants could be analysed (Gonzalez-Aguilera et al., 2008).

In Thp1Δ mutants the mutator action of the human enzyme AID is hyper-stimulated. This enzyme is known to generate mutations at specific loci in human B cells, where is important for antibody diversification. AID acts *in vitro* on ssDNA and induces mutations and recombination, which was observed in TREX-2 mutants but not in transcription mutants (Gonzalez-Aguilera et al., 2008). Interestingly, mutants of the TREX-2 complex showed transcription elongation defects through high G+C content genes, which were only observed *in vivo*, and not with *in vitro* systems (Gonzalez-Aguilera et al., 2008). This suggested that the physical association of the TREX-2 complex with the nuclear pore, and the connection between transcription and export is the driving cause of the phenotype observed, which is then rescued *in vitro* cellular extracts where the coupling is disrupted. This suggested that a failure in mRNA export, rather then a failure in transcription, would be the cause of genomic instability in yeast cells.

All these data showed that TREX-2 is involved in the maintenance of genome integrity in yeast, independently on SAGA, although the common subunit Sus1. Thus it was proposed that failure in coupling transcription with export, exerted by TREX-2, would cause accumulation of suboptimal mRNA molecule that could interact with the double helix of DNA from the *locus* where they are produced. This interaction DNA-RNA would leave one of the two DNA strands as ssDNA and the accumulation of ssDNA would increase

recombination and genomic instability, demonstrated also from the hyper-activation of human AID in TREX-2 mutants. Thus ensuring the correct coupling transcription-export would protect the yeast genome from genomic instability (Figure 15) (Gonzalez-Aguilera et al., 2008).

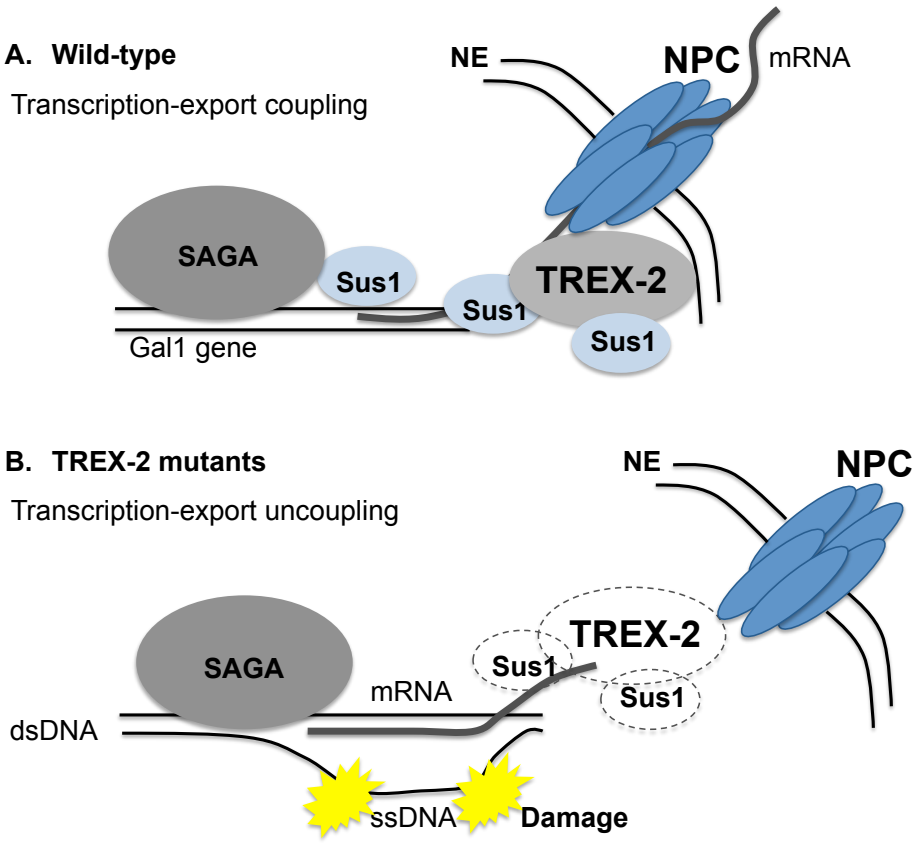

Figure 15 Genomic instability in TREX-2 mutants

Genomic instability in TREX-2 mutants is due to transcription-export uncoupling (A) In WT cells the concomitant action of SAGA and TREX-2 through sus1 stimulates the association of actively transcribed genes to the NPC for immediate export. (B) In TREX-2 mutants the export process is abolished thus creating an accumulation of RNA species in the double helix of DNA. Accumulation of ssDNA, through the displacement of one strand of the DNA, induces genomic instability in yeast TREX-2 mutants.
NE = Nuclear Envelope; NPC = Nuclear Pore Complex

1.5.5 TREX-2 and SAGA: a human perspective

To investigate the role of the human TREX-2 complex in genome stability in human cells first of all it is important to understand the numerous differences between yeast and humans in this context. As mentioned before the role of the yeast TREX-2 complex in genomic stability maintenance is connected with its role in gene gating. However the gene gating mechanism seems to be a prerogative of yeast and *C. elegans* (Rohner et al., 2013) while in mammalian cells, genes repositioning involves rather a movement from the

periphery to the nucleoplasm for transcriptional activation and it was observed in developmentally regulated genes (Ragoczy et al., 2006; Takizawa et al., 2008; Williams et al., 2006). Transcription, mRNA maturation and export are spatially and temporarily separated processes in mammalian cells. Thus the accumulation of mRNA in the nucleus in the case of mRNA export defects is unlikely to be associated with retention of RNA molecules in the double helix of DNA. Although there are evidences of formation of RNA/DNA hybrids (so called R-loops) also in mammalian cells, they have not been associated with export defects but rather with transcription and RNA processing defects or naturally occurring in key cellular processes (Wickramasinghe and Venkitaraman, 2016; Yu et al., 2003).

Moreover although TREX-2 is a highly conserved complex, the scaffold subunit of the mammalian complex is a protein of almost 2000 aminoacids, which harbours additional functional domains that are absent in the yeast Sac3, thus suggesting that the human TREX-2 might have additional functions other than mRNA export.

In addition, in yeast, TREX-2 and SAGA are connected, not only by the sharing of ySus1 but additional subunits of both complexes have been found to interact (Kohler et al., 2008; Rodriguez-Navarro et al., 2004). As mentioned before, in mammals, ENY2 represents a common subunit but not a physical link between the two complexes; indeed MS analyses have not been able to detect any other interaction (Umlauf et al., 2013). This might suggest more separated functions of TREX-2 and SAGA in human cells. However ENY2 in humans has been recently suggested to interact with additional complexes (Atanassov et al., 2016; Li et al., 2016) whose functions are probably not totally discovered (described hereafter). These additional interactions might add multiple layers of complexity to the role of the human TREX-2 in DNA repair being ENY2 an essential adaptor subunit and its distribution among complexes needs to be tightly regulated.

In the following sections I will describe in details the major subunit of the human TREX-2 complex (GANP) and the shared subunit between hSAGA and hTREX-2 (ENY2).

1.6 TREX-2-component Germinal Center Associated Nuclear Protein (GANP)

The main representative of the function, structure and localization of the TREX-2 complex is the human homologue of yeast Sac3, GANP. Although GANP is ubiquitously expressed in a variety of somatic cell types the name derives from its first discovery in 2000 in B cells of Germinal Centers (GC) (Kuwahara et al., 2000). Numerous findings have elucidated the role of GANP in immune cells, where it was found to be overexpressed, however the different domains of GANP point to a broader role of the protein in different cell types.

The *ganp* gene (also MCM3AP in human) encodes at least 6 transcripts variants and its located on chromosome 21. The full length GANP transcript is encoded by 28 exons. In 1998 it was identified a protein interacting with the minichromosome maintenance protein (MCM) 3 and it was shown to encode for an 80 kDa protein, named MCM3AP (Takei and Tsujimoto, 1998). The full-length transcript of the same locus was then shown to encode instead for a 210 kDa nuclear protein, which was overexpressed in GCs (Kuwahara et al., 2000). Subsequent northern blot and western blot analyses in B cells failed to identify the short form MCM3AP suggesting that it might be expressed at much lower levels than the full length GANP protein (Abe et al., 2000). it was shown, later on, that although MCM3AP and GANP are encoded from the same gene, they are indeed two different proteins, occupying different locations in the cells and transcribed from different promoters (Wickramasinghe et al., 2011). The scaffold subunit of the TREX-2 complex is represented by the full length GANP, and no evidences until now point for overlapping roles between GANP and MCM3AP.

1.6.1 Structure of GANP

The N-terminal domain of GANP, from aminoacid 1 to 400, is homologous to the classical FG repeat structure of proteins part of the NPC (Figure 16). This structure is highly similar between humans, mice, rats, rabbits, cows and sheeps, while in lower eukaryotes is missing from the homologous protein Sac3. The presence of FG repeats in the GANP protein suggests that it might have similar properties to nucleoporins.

GANP possesses also a RNA recognition motif, in the N-terminal side region, consistent with its role in mRNA export (Figure 16).

The Sac3 homology domain spans a region from aminoacid 686 to aminoacid 910 and the CID region from aminoacid 1162 to aminoacid 1256 (Figure 16). Structural studies and pull-down assay revealed that these regions, both in yeast and human, are necessary to bind ENY2 and Centrins *in vitro* (Jani et al., 2012).

Differently from Sac3, the C-terminal region of GANP contains a HAT domain (Singh et al., 2013; Takei et al., 2001). Recombinant HAT domain of GANP was shown to possess *in vitro* acetyltransferase activity toward H1 and H3, and it was shown *in vivo* with overexpression or KD experiments to control the levels of histone acetylation at the IgV locus in B cells. The C-terminal domain of GANP, as mentioned before, is identical to the 80 kDA MCM3AP protein, and the HAT domain resides in this region. This protein was suggested to interact and acetylate *in vitro* MCM3 (Takei et al., 2001; Takei and Tsujimoto,

1998). Initially it was suggested that the interaction between GANP and MCM3 could be involved in cell cycle regulation but further studies did not clarify the mechanism.

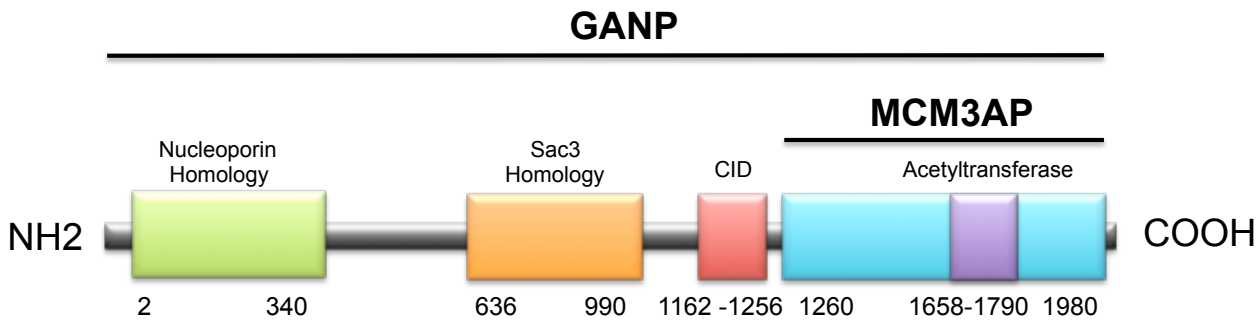

Figure 16 Schematic representation of the GANP protein

The different domains are depicted in boxes. The full-length GANP protein is constituted by 1980 aminoacids. The C-terminal domain is 100% identical to the protein MCM3AP, that is transcribed from a promoter lying in a GANP intron. NH2: N-terminal domain; COOH: C-terminal domain. Numers represent the aminoaacid position. Adapted from: (Wickramasinghe et al., 2010).

1.6.2 Role of GANP in B cells

GANP was found to be overexpressed in the GCs of immunized mice thus suggesting a specific role in immune response. GCs are indeed the sites where B cells proliferate and maturate to become mature plasma B cells that actively secrete antibodies for the efficiency of the "adaptive" immune response. The adaptation results in the fact that antibodies can be changed to recognize different pathogens, and this adaptation has genetic basis. In fact, antibodies genes (i.e. Ig genes) can be rearranged using CSR or Somatic Hypermutation (SHM), whether the constant or the variable region of the antibody is mutated. CSR requires induction of DSBs, activation of the DDR and subsequent repair by NHEJ in the Ig gene. Important step for the CSR is the induction of ssDNA nicks, exerted, among others, by the AID enzyme, at a conserved nucleotide motif called switch region.

As mentioned before it was suggested that GANP/MCM3AP associates with replication protein MCM3 and that possesses a DNA-primase domain at its N-terminal side regulated by phosphorylation on serine 502 selectively in stimulated B cells (Kuwahara et al., 2001) suggesting its involvement in the acceleration of B cell proliferation after immunization.

Role of GANP in CSR

GANP was shown to physically interact with AID in COS-7 cells (Maeda et al., 2010) although no specific interaction domain has been found so far, it was speculated the

interaction to occur through attractive forces generated by the opposite charges of the two proteins (Sakaguchi and Maeda, 2016). GANP was shown to be involved in the translocation of AID in the nucleus (Maeda et al., 2010), and in the recruitment of AID to the site of rearranged Ig locus (Singh et al., 2013).

In addition in Ramos B cells GANP role in IgV diversification was found to be dependent on its HAT activity. It was shown that overexpression of GFP tagged version of GANP significantly decreased Micrococcal Nuclease (MNase) sensitivity of the specific IgV locus analysed, and conversely depletion of GANP by siRNA caused a higher MNase resistance (Singh et al., 2013). Moreover ChIP of GFP-GANP and endogenous GANP showed that it interacts with the IgV locus in Ramos B cells and that this interaction is dependent on the HAT domain (Singh et al., 2013). Histones ChIP analyses at the IgV region showed that overexpression and KD of GANP respectively increases and decreases the levels of H3 acetylation at specific regions suggesting a role for GANP in the chromatin organization of antibodies diversification locus in human Ramos B cells (Singh et al., 2013). Other than its role in histone modification and the targeting of AID at the IgV locus, it was further shown that GANP plays a role in the repair of the IgV locus in chicken DT40 cells after AID action (Eid et al., 2014).

GANP is important to regulate the choice between HR and NHEJ favouring HR and abrogating NHEJ, in chicken B cells, where the protein is overexpressed and that this mechanism is due to GANP interaction with DNA-PKcs (Eid et al., 2014). In damage conditions GANP association with DNA-PKcs would hinder its NHEJ repair capacity (Eid et al., 2014). Nevertheless these results mainly pointed to a role of GANP in Ig diversification in a highly specialized system as bird B cells and at a very specific locus (i.e. IgV diversification locus) although colony survival assay in DT40 cells showed that further overexpression of GANP rendered cells less sensitive to campthotecin, suggesting a genome-wide effect on promoting HR (Eid et al., 2014).

However contrasting results were previously obtained in MEFs, where lack of GANP stimulated HR. In fact it was shown that lack of one GANP allele induced hyper-recombination, measured with a recombination substrate reporter vector and also in integrated reporter construct (Yoshida et al., 2007), recapitulating the phenotype observed before in yeast Sac3Δ strains (Gallardo et al., 2003). This suggests that GANP might have different roles accordingly to the model system, the cell type and the organism.

Interestingly any attempt of creating a GANP KO failed, both in mice (Sakaguchi et al., 2011) and chickens (Eid et al., 2014), while mice lacking GANP selectively in B cells (B-

GANP -/-) grew normally, showing only a delayed GC formation after immunization (Kuwahara et al., 2004) suggesting that GANP might have broader role in the organism other than Ig diversification.

1.6.3 Role of GANP in mRNA export: a shuttling matter

From its discovery in 2000 many reports investigated the role of GANP in B cells, but it was only in 2010 that the studies were extended to its role in human somatic cells and its role in mRNA export was discovered (Wickramasinghe et al., 2010). This and later reports showed that GANP depletion induces nuclear accumulation of mRNA species and is mainly localized at the nuclear periphery (Umlauf et al., 2013; Wickramasinghe et al., 2010). However there is still discordance whether GANP would shuttle between the nuclear interior and the periphery or it would be an integral member of the NPC. Contrasting results were obtained in this context. In support of the shuttling theory it was shown, by immunofluorescence experiments using antibodies against GANP, that the use of the transcription elongation inhibitor 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) would decrease GANP signal at the pore and create punctuate GANP signal in the inner nucleus, meaning that without transcription and no need for mRNA export, GANP would not need to reach the NPC to facilitate export (Wickramasinghe et al., 2010). In addition it was shown that GANP interacts with the mRNA export factors NXF1, which is located in the inner nuclear environment and is involved in earlier stages of export and that GANP depletion affects NXF1 localization at the nuclear pore (Wickramasinghe et al., 2010).

However more recent data showed that GANP interaction with the NPC is not affected by transcription inhibition using transcription inhibitors actinomycin D and α-amanitin, with or without permeabilization of the cells prior to fixation and that depletion of GANP does not affect NXF1 location at the periphery (Umlauf et al., 2013). In any case it is general accepted that GANP is, at least mainly, located at the NPC, in a manner dependent on basket nucleoporins, TPR and NUP153, and that it is required for mRNA export. The role of GANP in mRNA export was further investigated to show its involvement in the export of a subset of nuclear transcripts, only partially overlapping with NFX1-dependent transcripts (Wickramasinghe et al., 2014).

Given the association of TREX-2 with the NPC and the reports suggesting that it might exchange between nucleoplasm and periphery, live cell experiments were performed to asses the dynamicity of the complex. Fluorescence Recovery After Photobleaching (FRAP) experiments showed that ENY2-GFP and PCID2-GFP recovery after specific

bleaching of the NPC pool was extremely slow, 10 minutes for PCID2 and 8 hours for ENY2, reaching the recovery time observed for basket nucleoporins (10 minutes for NUP153) (Umlauf et al., 2013). These results, together with the observation that TREX-2-NPC association is independent on transcription pointed to behaviour of the TREX-2 complex that is similar to that of basket nucleoporins (Umlauf et al., 2013).

A plant exception

TREX-2 role in mRNA export is highly conserved among eukaryotes, and a functional TREX-2 complex also exists in plants in which two homologs of ySac3, SAC3B and SAC3A are potential members of the complex (Lu et al., 2010) (Figure 17). Both SAC3s interacts with *Arabidopsis* THP1 and *Arabidopsis* Centrins 1 and 2, but while SAC3A exhibits nucleoplasm localization, SAC3B is mainly located at the nuclear periphery (Lu et al., 2010). Interestingly none of the SAC3 proteins is involved in mRNA exports in plants. Mutation in SAC3B causes genome wide transcription alteration, that is dependent on an increase of dimethylation of K9 of histone H3 (H3K9me2), a heterochromatin mark (Yang et al., 2017). This phenotype was also observed in THP1 mutants pointing to a role for plants TREX-2 in preventing transcriptional silencing. It was speculated that gene gating might happen in plants and that the tethering of the transcribed chromatin at the nuclear pore through TREX-2 might be involved in maintaining the active transcriptional status (Yang et al., 2017). However it is important to mention that SAC3B does not contain the HAT domain found at the C-terminal region of GANP, that could have explained heterochromatin spreading, through loss of histones acetylation. However further studies are necessary in this direction to understand the role of plants Sac3 proteins.

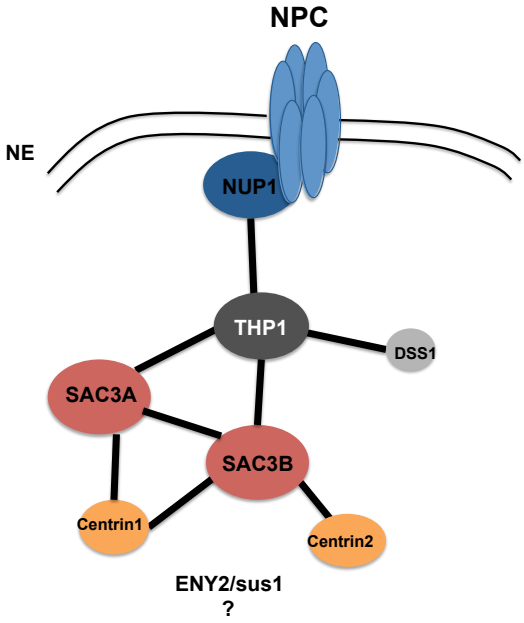

Figure 17 Schematic representation of *Arabidopsis* TREX-2 complex

SAC3A and SAC3B are bona fide components of the TREX-2 complex in plants; they are both present in the complex and they interact with THP1 and centrins. Black lines depict the validated interactions. No interaction has been found yet between the SAC3 proteins and *Arabidopsis* ENY2. Adapted from (Yang et al., 2017)

1.6.5 GANP-associated diseases

As mentioned earlier GANP is described to be specific overexpressed in B-cells where is important for their maturation in GCs and mRNA export. However, it was lately found that mutations in the *GANP* gene are associated with complex-phenotype diseases that point to a broader role for GANP in DNA repair. Whole exome sequencing was performed on a patient that showed immunodeficiency, genomic instability, skin changes and myelodisplasia, and mutations in two genes were found, one of them being MCM3AP/GANP. A heterozygous nucleotide exchange in the exon 2 of *GANP* gene mutates the Proline (P) in position 443 into a Serine (S) (P443S). Interestingly a twofold accumulation of the full-length GANP mutated protein was observed into patient-derived lymphoblasts, while showing at the same time a two-fold downregulation of the *MCM3AP* gene product, transcribed from a promoter within a GANP intron (Gatz et al., 2016). Interestingly, DR-GFP based approach, revealed that patient-derived lymphoblasts showed a reduction in HR-mediated repair, while NHEJ was not significantly affected. Overexpression of the WT GANP in the patient's lymphoblast increased the HR efficiency in the patient's cells, while it decreased it in the father's cells (Gatz et al., 2016) and patient-derived fibroblasts showed an increase in γH2AX and 53BP1 foci, consistent with repair defects (Gatz et al., 2016). The complex phenotype can be also associated with the

second mutation in the *POMP* gene (part of the 19S proteasome subunit), but the fact that overexpression of WT GANP in patient's cells reduced the amount of 53BP1 foci and rescued the HR defects seems to confirm a role for GANP in the repair-defect associated phenotype (Gatz et al., 2016) although the exact mechanism is still unclear. In fact, another subsequent study revealed that different GANP mutations in several patients with intellectual disability disorder lead to a 20% depletion of the GANP protein, although no DNA repair defect could be associated after exposure to UV or ionising radiation, suggesting that GANP might not have a role in radiation-induced DNA damage repair (Schuurs-Hoeijmakers et al., 2013). However considering the amount of previous reports pointing to a role of GANP in DNA-repair it cannot be excluded its involvement in specific repair pathways. Moreover, GANP expression was found to be impaired in human breast cancer samples, and mice with mammary gland-targeted GANP depletion developed breast cancer in one year after pregnancy; both homo-deficient and hetero-deficient GANP mice (Kuwahara et al., 2016). This increased susceptibility to solid tumors of GANP-depleted mice, and the reduced expression of GANP in actual patients, was speculated to be connected with the resistance to develop solid tumors of Down Syndrome (DS) patients, who have three copies of the chromosome 21, where *GANP* gene is located, suggesting GANP as a novel tumor-suppressor gene. There are no evidences of increased GANP levels in cells with trisomy 21 and further studies are needed in this direction, however, to make this theory a likely scenario there is also the evidence that overexpression of GANP is associated with high incidence to develop lymphomagenesis (Fujimura et al., 2005), another feature characteristic of DS patient. Further studies are necessary to investigate at which stage of tumor development GANP might act and of course the underlying molecular mechanisms.

1.7 Human Enhancer of Yellow (ENY) 2: a crucial adaptor

GANP major partner in the TREX-2 complex is ENY2. ENY2 is a very small protein harbouring no functional or catalytic domain, however is fundamental for TREX-2 stability and activity because, as mentioned before, is required to stabilize the long and unstable α-helix of GANP. The other major ENY2-containing complex is the SAGA DUB, which also relies on this small protein to be stabilized. In the DUB module ENY2 functions as an adaptor, together with ATXN7L3, fundamental for the proper structural organization of the entire module and is necessary for its function both in yeast and mammals (Samara et al., 2010; Zhao et al., 2008). The deubiquitinase enzyme in the DUB module is represented by USP22 (Zhang et al., 2008); however the function of USP22 is strictly dependent on the

proper organization of the DUB module. In addition ATXN7 has the function of anchoring the USP22-ATXN7L3-ENY2 complex to the rest of the SAGA complex.

Very unexpectedly it has been reported that depletion of USP22 is not accompanied by an increase in H2Bub1 levels, as instead is the loss of ENY2 or ATXN7L3 (Atanassov et al., 2016). The fact that loss of the adaptor proteins has a greater effect on H2Bub1 compared to the loss of the catalytic subunit suggested that these adaptors might stabilize other DUBs. In fact it has been shown that in human cells other two possible USPs exist that associate with both ENY2 and ATXN7L3 to form a functional DUB module, USP27X and USP51 (Atanassov et al., 2016). It has been shown that these additional DUBs do not interact with SAGA, given the absence of ATXN7 in the complex (Figure 18D, E). USP27X and USP22 share 82% of identity, while USP51 an USP22 share 70% of identity, they both contain a NLS and they require both ENY2 and ATXN7L3 for their catalytic activity *in vivo* and *in vitro* (Atanassov et al., 2016). The activity of USP22, USP27X or USP51 is regulated by the availability of ENY2 and ATXN7L3, thus upon USP22 depletion the interaction between ATXN7L3 and USP27X or USP51 is enhanced. This would explain why ablation of USP22 does not result in increased H2Bub1 levels as ablation of the adaptor proteins does.

These data show that ENY2, although being a small protein, is fundamental for the activity of other proteins functioning as adaptor protein. Moreover, as mentioned before, USP51 has been implicated in the DDR, suggested to deubiquitinate H2AK13/15ub at damage sites. It has been proposed that USP51 levels have to be tightly regulated for proper DNA repair (Wang et al., 2016).

This report, together with the discovery of a functional DUB module containing USP51, ENY2 and ATXN7L3, raises new questions about the role of ENY2 in DNA repair and its regulation. It might be possible that the function of USP51 in DNA repair is regulated by ENY2 and ATXN7L3 availability. However further studies are necessary in this direction to asses the role of newly-discovered DUBs in DNA repair.

In addition to USP27X and USP51-containing DUBs, ENY2 has been found associated in the cytoplasm with another small protein, arose from a retro-transposition of ATXN7L3 and thus called ATXN7L3B (Li et al., 2016). The well-known ENY2 partner, ATXN7L3, harbours a Zinc Finger (ZnF) domain that is required for DUB module binding to DNA (Kohler et al., 2010). Cytoplasmic ATXN7L3B lacks this domain and does not associate with SAGA components, however its depletion or overexpression can alter H2Bub1 levels on chromatin through release or sequestration of ENY2 (Li et al., 2016). The interaction

between ATXN7L3B and ENY2 has been suggested to be important for ENY2 distribution between cytoplasm and nucleus, and thus ENY2 availability for the SAGA DUB, having as an indirect effect the regulation of H2Bub1 deubiquitination (Li et al., 2016).

All these data together suggest that ENY2 is a key subunit of several complexes and that regulating its distribution among them might represent a way to control different key cellular processes (such as mRNA export, transcription and DNA repair).

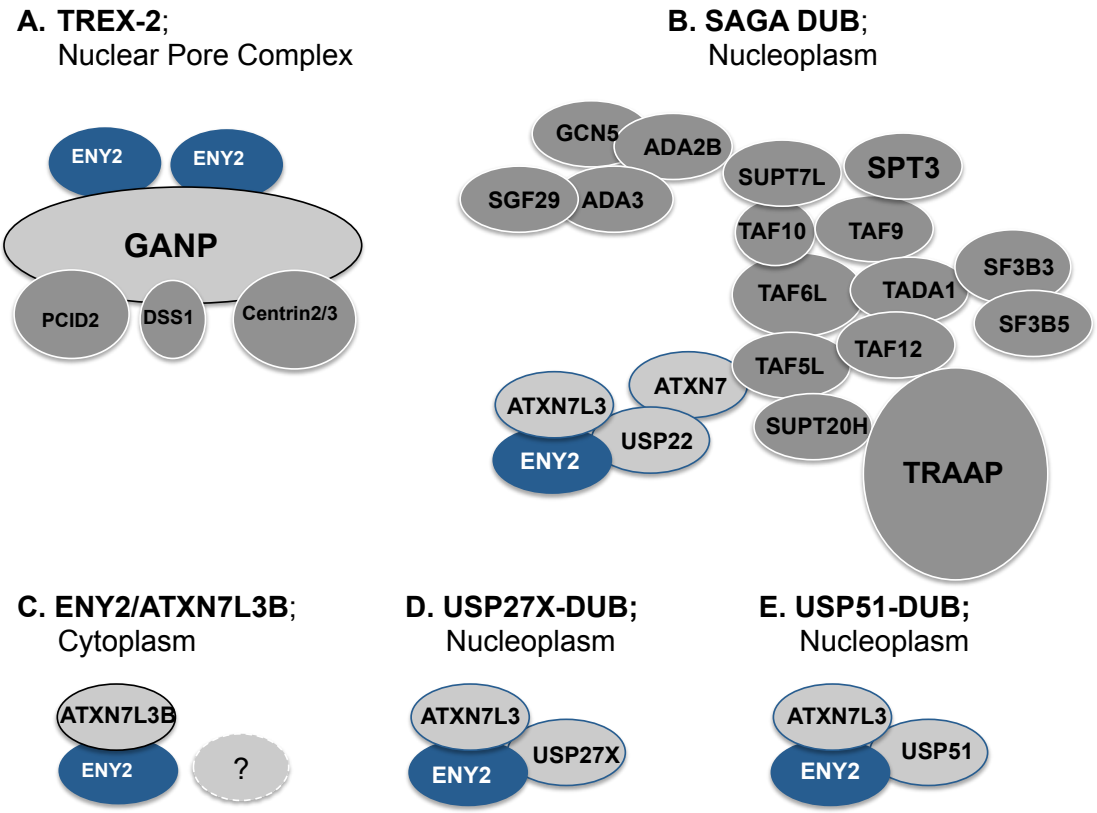

Figure 18 Different ENY2-containing complexes in human cells

The best-characterized ENY2 containing complexes are (A) TREX-2 localized at the NPC and (B) the SAGA DUB module, localized in the nucleoplasm. Recently, additional ENY2 partners have been discovered. (C) ENY2 interacts with ATXN7L3B in the cytoplasm. (D-E) Additional DUBs have been discovered in human cells, containing either USP27X (D) either USP51 (E), but both requiring ATXN7L3 and ENY2 for their deubiquitinase function. These additional DUBs do not contain ATXN7 thus they do not interact with SAGA.

2. AIM OF THE WORK

The maintenance of proper genetic information is essential to ensure correct cellular functions and to avoid genomic instability that is a hallmark of cancer. Exogenous and endogenous sources cause approximately 70 thousand molecular lesions per day on the DNA.DSBs are among the most dangerous types of lesions. If unrepaired they can lead to genomic rearrangements and eventually cancer. For this reason DSB activate a highly specialized pathway in the cell called the DDR that relies on a complex signalling cascade. Histones PTMs and the chromatin environment play an essential role in DNA repair. Moreover recently it has been shown that proteins part of the Nuclear Pore Complex (NPC) and proteins that associate with it, although not directly recruited at the sites of damage, play an important role in DNA repair both in yeast and in mammalian cells, showing to have conserved roles.

TREX-2 is a highly conserved complex among eukaryotes that physically associates with the NPC and contributes to the export of mRNA species from the nucleus to the cytoplasm in a process that involves tethering of the transcribed chromatin at the NPC (gene gating). The connection between transcription and export in yeast is achieved through the physical interaction of TREX-2 with SAGA. This connection has been shown to be required for the maintenance of genome integrity as mutants of the TREX-2 complex, but not SAGA mutants, show hyper-recombination phenotype, a hallmark of genomic instability.

In mammals TREX-2 and SAGA share one subunit, present in both complexes, but since there are no evidences of a gene gating mechanism that would connect actively transcribed regions to the NPC the interaction between TREX-2 and SAGA in mammals is limited.

The high degree of homology in the composition and structure of the TREX-2 complex suggests also a similar functionality. Indeed the TREX-2 complex has been shown to be involved in mRNA export also in human cells. Nevertheless the scaffold subunit of the human complex, GANP, although showing homology with the yeast counterpart, contains additional functional domains, that might point to different roles in humans.

The aim of my PhD was to investigate the potential role of the human TREX-2 complex in DNA repair and a potential interplay between TREX-2 and SAGA in this context.

To this end we used biochemistry and microscopy approaches to analyse DNA repair in HeLa and U2OS cells depleted of the TREX-2 scaffold subunit GANP. The depletion of the shared subunit between TREX-2 and SAGA let us disclose interplay between the two complexes in human cells important for DNA repair.

In addition we investigated possible additional role for human GANP in the context of global chromatin epigenetic status.

3. RESULTS

Using biochemical and microscopy approaches we analysed the role of the human TREX-2 complex in DNA repair in human cells. We discovered an interplay between TREX-2 and the SAGA DUB module required to control H2Bub1 levels in response to DNA damage. These results are part of a manuscript entitled "A regulated interplay between the human TREX-2 complex and the SAGA DUB module is required for efficient DNA damage repair" (Section 3.1). Our results suggest that the activity of SAGA DUB must be tightly regulated to maintain H2B/H2Bub1 balance important for DNA repair. One level of regulation could come from the TREX-2 and SAGA common subunit ENY2. ENY2 is a highly unstable protein required for the stability of the TREX-2 complex and the activity of the DUB module. We investigated how, in DNA damage conditions, ENY2 levels and distributions are regulated. These results are shown in separate sections, however they are connected with the role of TREX-2 and SAGA DUB in DNA repair.

Finally, additional experiments pointed out a role for the human GANP in regulating the global chromatin status; these experiments are shown in the last paragraph of the Results section.

3.1 A regulated interplay between the human TREX-2 complex and the SAGA DUB module is required for efficient DNA damage repair

In our manuscript we report a novel role for the human TREX-2 complex in DNA repair. Cells depleted of TREX-2 scaffold subunit GANP accumulate unrepaired global DNA damage and have delayed breaks resolution. We show that GANP/TREX-2 is required for efficient repair via HR and that this is due to a decrease in H2Bub1 global levels. H2B/H2Bub1 balance is regulated by the concomitant action of RNF20/40 and the SAGA DUB. We add a new piece of data in this regulation showing that GANP/TREX-2 controls DUB activity and that this is fundamental for correct DSBs' repair through HR.

A regulated interplay between the human TREX-2 mRNA export complex and the SAGA DUB module is required for efficient DNA damage repair

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Running title: Opposite effects of TREX-2 and DUBs in repair

Key words: Homologous Recombination, DNA repair, GANP, ENY2, ATXN7L3, RAD51, SAGA complex, USP22, γH2AX, double strand break (DSB), DNA damage response (DDR).

Abstract

DNA repair is critical to maintain genome integrity and its dysfunction can cause accumulation of unresolved damage that leads to genomic instability. The Spt-Ada-Gcn5 Acetyltrasferase (SAGA) coactivator complex and the nuclear pore-associated TRanscription and EXport complex 2 (TREX-2) couple transcription with mRNA export. Here we identify a novel interplay between human TREX-2 and the deubiquitination module (DUBm) of SAGA, required for genome stability. We find that the scaffold subunit of TREX-2, GANP, positively regulates DNA repair through homologous recombination (HR). In contrast, DUBm adaptor subunits, ENY2 and ATXNL3, are required to limit unscheduled HR. These opposite roles are achieved through monoubiquitinated histone H2B (H2Bub1). Interestingly, the activity of the DUBm of SAGA on H2Bub1 is dependent on the integrity of the TREX-2 complex. Thus, we describe the existence of a functional interaction between human TREX-2 and SAGA DUBm that is key to maintain H2B/HB2ub1 balance needed for efficient repair and HR.

Eukaryotic cells are constantly exposed to DNA damage that can arise from exogenous and endogenous sources [1-3]. Among the plethora of different lesions that can be generated on the DNA molecules, double stranded beaks (DSBs) are among the most dangerous type of damage, because if not successfully repaired, they can lead to chromosomal rearrangements [4, 5]. The DNA damage response (DDR) is a complex signalling cascade that is activated by DSBs that are either exogenously generated by ionizing radiation, or by chemicals and by programmed DSBs that happen in well-defined locations in the genome, such as in meiosis or at immunoglobulin class switch recombination (CSR) locus [6].

The main hallmark of the DDR is the phosphorylated form of the histone variant H2AX on Serine 139 (γH2AX) deposited by the activated ATM kinase [7]. Two main DNA repair sub-pathways exist: non-homologous end joining (NHEJ) and homologous recombination (HR). While the former ligates the extremities of the two broken ends and can often leave deletions at the site of the break, HR restores in a faithful way the genetic information at the site and in the vicinity of the break, using the homologous chromatid as a template for repair, thus being by definition error-free. HR takes place in S and G2 phases of the cell cycle [8], given the availability of the sister chromatids to be used as a template, while NHEJ can be active throughout the cell cycle. For HR to occur, the key step is the production of a 3' single stranded (ss) DNA end through the process of resection [9] that depends on specific cell-cycle regulated proteins [10-12] thus, being active in S and G2 phases.

The TRanscription and EXport complex-2 (TREX-2) complex is involved in mRNA export [13-19]. The human TREX-2 complex is composed of five subunits, GANP, ENY2, PCID2, Centrin2/3 and DSS1 (which in yeast are: Sac3, Sus1, Thp1, Cdc31 and Sem1, respectively). Moreover, it has recently been shown that hTREX-2 stably associates with the Nuclear Pore Complex (NPC) and that this interaction is crucial for its role in mRNA export [17].

The Spt-Ada-Gcn5 Acetyltrasferase (SAGA) coactivator complex and the TREX-2 complex are evolutionarily conserved among eukaryotes. SAGA is composed of 19 subunits organized in

several functional modules with different activities and roles [20]. The histone acetyltransferase (HAT) module of SAGA is required at gene promoters to induce acetylation of Lysine (K) 9 of histone H3 (H3K9ac) and H3K14. This activity is catalysed by the GCN5 enzyme [21]. The deubiquitination module (DUBm) of SAGA is required to remove mono-ubiquitin from K120 of human H2B (H2Bub1) in gene bodies [22-24]. These activities are required for the transcription of all active genes both in yeast (y) and human (h) cells [25, 26]. In human cells, monoubiquitination of histone H2B, is deposited by RNF20 and RNF40 [27] and deubiquinated by SAGA [22-24]. Interestingly, H2B in mammalian cells was found to be monoubiquitinated in response to DNA damage and is necessary for efficient DSB repair [28, 29].

TREX-2 and SAGA share one subunit, ySus1/hENY2 [30]. In TREX-2 ySus1/hENY2 binds to the scaffold protein ySac3/hGANP in two copies [31]. In SAGA, hENY2 binds to ATXN7L3, and together with ATXN7 and the DUB enzyme, USP22, forms the DUBm [32, 33]. Moreover, in human cells, ENY2 and ATXN7L3 form alternative DUBm complexes with the deubiquitinating enzymes USP27X, or USP51, which are both 70-80% identical to USP22. These alternative DUBm members lack ATXN7 and thus, do not associate with SAGA. Nevertheless they can deubiquitinate H2Bub1 *in vitro* and *in vivo* [34]. It has been shown that the knock-down of ENY2 destabilizes hGANP in TREX-2 and ATXN7L3 in the SAGA DUBm [17, 35]. Moreover, the activity of the SAGA DUBm and the above-mentioned alternative DUBm-s strictly require the adaptors ENY2 and ATXN7L3 for their activity [34]. For simplicity, hereafter the three SAGA related DUBm-s will be called USP22-related DUBm-s.

In yeast, the physical and functional interaction between TREX-2 and SAGA complexes was described and was suggested to connect on-going transcription with the NPC for immediate RNA export in a process called "gene gating" [30, 36-40]. Destroying this connection at the export level has been shown to induce genome instability, as yTREX-2 and yNucleoporins (Nup) mutants, but not ySAGA mutants, show hyper-recombination phenotype [41-44].

In mammalian cells the involvement of Nups and associated proteins in the DDR has not been connected with RNA export or transcription defects [45-47]. Moreover, the interplay of mammalian TREX-2 or the SAGA DUBm has not been studied in the context of DNA repair.

Here we have investigated the role of TREX-2/GANP and the SAGA DUBm and related DUMm-s in DSB repair. We find that loss of the scaffold subunit of TREX-2 (GANP) results in DNA repair deficiency by HR. This GANP-dependent HR defect is accompanied by decreased resection and is mediated by down-regulation of H2Bub1 levels. In contrast, down regulation of the DUB activities (by knockdown of either ENY2 or ATXN7L3) results in upregulation of HR efficiency and a parallel increase in H2Bub1 levels. Interestingly, concomitant depletion of TREX-2 and the DUB activity restores the HR pathway, RAD51 recruitment to DNA damage sites and results in normal H2Bub1 levels upon DNA damage. These results together demonstrate a functional cross-talk between human TREX-2 and the USP22-related DUBm-s that is important for correct DSB repair during HR.

Results

Depletion of the TREX-2 scaffold protein, GANP, affects the efficiency of DNA repair

In order to investigate the role of the TREX-2 complex in genome stability in mammalian cells, we performed clonogenic survival assay in HeLa cells treated with the radiomimetic drug phleomycin in combination with siRNA-mediated knockdown of GANP. Interestingly, cells depleted of GANP displayed a significant increase in sensitivity to all concentrations of the drug compared to control cells (Fig. 1a). This result suggests a GANP-dependent defect in either the activation of DDR, or repair of DSBs. The efficient GANP depletion by siRNA was monitored by Western blot (Fig. 1b). Moreover, GANP depletion leads to substantially elevated γH2AX levels (Fig. 1b,c)

To test whether GANP depletion induces spontaneous DNA damage, we carried out a COMET assay on GANP-depleted cells in the absence of drug. Interestingly, GANP depletion leads

to increased comet-tails that are indicative of increased DNA breaks (Fig. 1d), further supporting the notion that GANP is key in maintaining genome stability as its absence leads to persistent DNA breaks in the absence of damaging agents.

To further investigate the role of GANP/TREX-2 in repairing DSBs, we induced DNA damage with neocarzinostatin (NCS) for 15 minutes and monitored the kinetics of repair by the appearance and disappearance of γH2AX and 53BP1 foci, in Hela and hTERT RPE1 control cells, or cells depleted for GANP. We used depletion of known DNA repair factors XRCC4 and SET as positive controls for delayed damage repair in both cell lines [48, 49]. As expected, cell treated with control siRNA showed a progressive reduction of both DDR marks (γH2AX and 53BP1) at 8 and 16 hours of repair (Fig. 2a,b). However, knockdown (KD) of GANP also delayed the disappearance of both γH2AX and 53BP1 foci after 8 and 16 hours of repair in HeLa and RPE1 cells, equivalent to the delay observed upon depletion of XRCC4 and SET (Fig. 2a,b and Supplementary Fig. 1).

Next, we analysed whether the depletion of GANP would affect the DDR activation in NCS treated cells. Our analyses show that in cells depleted for GANP, the initial recruitment of 53BP1 was not affected (Fig. 2b) and activation of ATM, monitored by its auto-phosphorylation on Ser1981, or by the phosphorylation of its substrate KAP1, was comparable to control cells (Fig. 2c). Interestingly however, we find that in GANP-depleted cells phospho-ATM and γH2AX were persistent 8 hours after NCS treatment suggesting that GANP is involved in repair of exogenously inflicted DNA breaks (Fig. 2c).

Since GANP/TREX-2 was shown to be located mainly at the inner side of the NPC [17], we wondered whether the remaining unrepaired breaks were accumulated at the periphery of the nucleus, or equally distributed between periphery and inner nucleus. Analysis of the raw intensity data for γH2AX showed no shift in the distribution of gH2AX in the two compartments between GANP-depleted cells and control cells, or cells depleted of XRCC4, suggesting a more global effect (Supplementary Fig. 1a). These results suggest that although TREX-2 is predominantly localized at the nuclear pores, it has a rather global role in DSB repair.

Depletion of GANP/TREX-2 affects the efficiency of homologous recombination

DSBs are repaired by two main repair pathways, NHEJ and HR. We showed that depletion of GANP results in unrepaired persistent breaks throughout the nucleus without affecting the early activation of the DDR, suggesting the involvement of GANP in repair. Thus, we next analysed the involvement of GANP in the two main repair pathways. To assess the rate of HR, we used a U2OS cell line stably integrating a DR-GFP reporter [50, 51], and to analyse NHEJ the GCV6 human fibroblast cell line [52]. Interestingly, depletion of GANP reduced the efficiency of homologous recombination by 60% (Fig. 3a). The reduced HR efficiency in GANP-depleted cells was almost as strong as that caused by the depletion of RAD51, a key player in homology-directed repair [53] (Fig. 3a). Consistently, HeLa cells depleted of GANP were more sensitive to camptothecin (Fig. 3b) that induces DSBs in S-phase [54]. HR is the preferred pathway in S and G2 phases of the cell cycle, thus a possible effect on cell cycle progression could explain the dramatic effect observed on HR efficiency. To verify this possibility, we analysed cell cycle progression either by propidium iodide incorporation in U2OS cells, or EdU incorporation in HeLa cells in GANP KD conditions. We did not observe any significant effect on cell cycle in both cellular systems depleted of GANP (Supplementary Fig. 2a,b), and GANP depletion did not affect RAD51 protein levels (Supplementary Fig. 2c). In contrast to the role of GANP in HR, we did not detect a significant change in NHEJ following GANP depletion (Fig. 3c). These experiments together show that GANP is required for promoting HR.

Resection is decreased in the absence of GANP/TREX-2

To dissect the steps of HR that are affected by GANP depletion and to test whether the above-described HR defect is accompanied by a decreased end resection, we analysed the recruitment of proteins involved in this process. To this end, we used U2OS cells stably integrating an ISceI site flanked the Lac operator (LacO) array and stably expressing the LAC repressor (LacI) fused to GFP that allows visualization of the locus [55]. Addition of doxycycline enables the expression of the ISceI enzyme and the induction of a single DSB at the lacO locus. Using microscopy, we analysed

the recruitment of a series of factors known to be involved in resection, or which serve as markers of resection in human cells, such as BRCA1, phospho-RPA, CtIP, and 53BP1 [56]. In agreement with our above results, depletion of GANP dramatically reduced recruitment of BRCA1, phosphorylation of RPA at different phosphorylation sites (serine 4/8 and serine 33) and recruitment of RAD51, as compared to control cells (Fig. 4a-d). BRCA1 in association with CtIP and the MRN complex forms the BRCA1 C-complex that is required for the early steps of resection [57, 58]. Thus, we also analysed recruitment of CtIP to further investigate whether homologous recombination is affected at the initial stages of resection (Fig. 4e). Recruitment of CtIP was also affected in GANP-depleted cells suggesting a defect in resection initiation (Fig. 4e). However, GANP depletion did not affect recruitment of the early DDR factor 53BP1 (Fig. 4e). Efficient induction of breaks was monitored by γH2AX (Supplementary Fig. 3a). The defect in RAD51 recruitment was also observed in GANP-depleted HeLa cells, treated with NCS (Fig. 5a,b). As observed before, there was no difference in 53BP1 recruitment (Supplementary Fig. 3b) between GANP-depleted and control cells. All these experiments together indicate that GANP/TREX-2 is required for efficient HR, and that the recruitment of resection factors to DSBs and DNA-end resection is impaired in the absence of GANP.

ENY2 and ATXN7L3 are both required to avoid unscheduled homologous recombination

To further investigate the role of the human TREX-2 complex in genome stability, we tested whether depletion of another subunit of the complex would have the same effect on DNA repair. To this aim, we depleted ENY2 that associates with GANP in two copies [31]. Surprisingly, in contrast to GANP depletion (Fig. 3a), which decreases HR efficiency by about 3-fold, siRNA depletion of ENY2 increased HR efficiency 2-fold as monitored by the DR-GFP assay (Fig. 6a). Consistently with the significant increase in the HR efficiency, clonogenic survival assay in HeLa cell carried out following ENY2 depletion showed a decrease in the sensitivity of cells to camptothecin, when compared to control cells (Fig. 6b), without affecting cell cycle progression (Supplementary Fig.

4a). These experiments suggest that ENY2 plays the opposite role when compared to GANP and thus seems to be required to suppress HR.

To understand if breaks are efficiently repaired in the absence of ENY2 we analyzed the kinetics of repair in asynchronous HeLa and RPE1 cells treated with NCS using γH2AX as a marker. ENY2 depleted cells, displayed persistent γH2AX at 16 hours after damage induction compared to control cells (Fig. 6c and Supplementary Fig. 4d). Moreover, ENY2 depleted cells, although being more resistant to camptothecin (S and G2 phases specific damage), were more sensitive to DSBs induced with phleomycin, compared to control cells (Supplementary Fig. 4b). These results suggest that the down-regulation of ENY2 creates conditions that would favor HR in an unscheduled way being potentially deleterious for the cells.

Interestingly, ENY2 or GANP depletion have opposite effects on DNA repair by homologous recombination. ENY2 is an integral component of TREX-2 and several USP22-related DUBm-s (see Introduction). To investigate whether the role of ENY2 in supressing HR can be ascribed to the DUBm-s, or to TREX-2, we performed the DR-GFP assay in cells depleted of another key subunit of the DUBm-s, ATXN7L3. ATXN7L3 depletion had the same effect as depletion of ENY2, suggesting that ENY2 suppresses HR as component of the USP22-related DUB modules (Fig. 6d and see also Fig. 6a). These experiments further suggest that hTREX-2 and the USP22-related DUB modules play opposite roles in DNA repair process.

TREX-2 depletion affects the global cellular levels of H2Bub1

The USP22-related DUB modules are involved in the removal of monoubiquitin from H2Bub1. While the recruitment of RNF20/40 catalysing the formation of H2Bub1 after DNA damage has been studied [28, 29, 59], little is known about the importance of H2Bub1 deubiquitination and the balance between histone H2B and H2Bub1 in the response to DNA damage. As formation of H2Bub1 is important for homology directed repair [29], an extreme increase in H2Bub1 levels upon DUB depletion could explain unscheduled HR. Therefore, we wondered whether the opposite effects of TREX-2 and DUBm-s in homology directed repair could

be due to different effects on H2Bub1 levels. We analysed H2Bub1 levels in HeLa cells depleted of either GANP or ENY2. Interestingly, GANP-depleted cells showed decreased global H2Bub1 (Fig. 7a). However, cells lacking ENY2 showed increased levels of H2Bub1, possibly as a consequence of the disruption of the DUB modules (Fig. 7a and see Introduction), further substantiating the opposite effects of GANP and ENY2, seen in the HR assay. Next, we treated cells with increasing concentrations of NCS. As shown before [28], the levels of H2Bub1 increase upon DNA damage in a NCS concentration-dependent manner (Supplementary Fig. 5a). In GANP-depleted cells, however there is a minor increase at the level of H2Bub1 upon DNA damage compared to control cells (Fig. 7b,d). ENY2 depleted cells on the other hand had elevated H2Bub1 levels even in the absence of DNA damage (Fig. 7a,b). These results suggest that the opposite effects observed on the HR/DDR might be due to the inverse influences of GANP in TREX2, or ENY2 in the USP22-related DUBm-s on the histone H2Bub1 mark.

TREX-2 is required to avoid excessive H2Bub1 deubiquitination by the USP22-related DUB modules in response to DNA damage

To investigate whether the observed decrease in H2Bub1 levels upon GANP depletion are due to an increased DUB activity, we tested whether the concomitant depletion of the three USP22-related DUBM-s, through ENY2, or ATXN7L3 KD, would restore normal H2Bub1 levels. The co-depletion of GANP and ENY2, or GANP and ATXN7L3, restored normal H2Bub1 levels when compared to H2Bub1 levels in mock siRNA treated undamaged or damaged cells (Fig. 7c,d,g). These results together suggest that the TREX-2 complex can negatively regulate the deubiquitination of H2Bub1, and that, if destabilized through GANP depletion, the activity of the DUB modules is enhanced. To further understand whether this negative effect of TREX-2 on H2Bub1 levels is important in DNA repair by HR we analysed the recruitment of RAD51 at the ISceI site in cells depleted of either GANP alone, or co-depleted of GANP and ENY2, or GANP and ATXN7L3. Depletion of GANP alone dramatically reduced the number of cells recruiting RAD51 to the LacO array after IsceI cleavage (Fig. 4d and Fig. 7e). However, the additional

depletion of ENY2 completely rescued the defect to control levels (Fig. 7e), as did the depletion of ATXN7L3 (Fig. 7f). Efficiency of double KD was monitored by RNA levels (Supplementary Fig. 5b,c). These results together indicate that TREX-2 integrity is required to avoid excessive H2Bub1 deubiquitination in response to DNA damage, and further suggest that the observed effects of ENY2 depletion are rather due to its role in the USP22-related DUB modules together with ATXN7L3.

Discussion

Here we have described a new functional interaction of the TREX-2 complex with the SAGA-related DUBm-s in DNA repair in human cells. Importantly our results indicate that the TREX-2 scaffold subunit, GANP, is required for efficient DSB repair in human cells, as GANP-depleted cells accumulate unrepaired breaks, as indicated by increased γH2AX signal and comet-tail moment, as well as having increased sensitivity to genotoxic agents. In good agreement, a previous study in human cells analysing the role of PCID2 and DSS1, two other TREX-2 subunits, showed that depletion of PCID2, or DSS1, also cause DNA break-accumulation as indicated by an increase in γH2AX staining and in comet-tail moment [60].

Here we further show that depletion of GANP/TREX-2 particularly affects the homologous recombination pathway. A prerequisite for homologous recombination is the production of a 3' ssDNA overhang in the process known as resection, which will invade the homologous duplex through RAD51. Consistent with a decrease in homologous recombination efficiency, GANP-depleted human cells (HeLa and U2OS) are defective in the early stages of resection, and subsequently in RAD51 loading both at the IsceI-induced break and genome wide (Fig. 4d and Fig. 5). Our data are in accordance with previous studies in chicken B cells where it was shown that GANP stimulates HR [61]. Note, however, that GANP was also shown to suppress hyper-recombination in mouse cells [62], suggesting that GANP/TREX-2 might have different roles depending on the origin of the cells studied.

The association of human GANP with the NPC depends on basket nucleoporins NUP153 and TPR [17]. Recent studies described a role for these nucleoporins in DDR, affecting primarily the nuclear import of 53BP1 and its sumoylation [45-47, 63]. However, we did not detect (i) any 53BP1 import defects in the absence of GANP/TREX-2 (Fig. 2b), (ii) any defects in the recruitment of 53BP1 at the Isce I-induced break (Fig. 4f), or (iii) defects in genome wide formation of 53BP1 foci upon NCS treatment (Supplementary Fig. 3b). Thus, the role of GANP/TREX-2 in global DNA repair is likely independent from its interaction with basket nucleoporins. Moreover, we did not detect specific accumulation of unrepaired breaks at the nuclear periphery (Supplementary Fig. 1b), further suggesting that TREX-2 acts through an NPC-independent mechanism.

Interestingly, the GANP/TREX-2 depletion reduced H2Bub1 levels either under steady-state or damage conditions. The fact that GANP/TREX-2 does not have any known E2/E3 ubiquitin ligase activity and the fact that the DUB module of SAGA is the major DUB responsible for the removal of ubiquitin from H2Bub1 in mouse ES cells [25] suggests that GANP/TREX-2 might affect histone H2Bub1 levels and consequently DNA repair by affecting the activity of the SAGA DUB module and potentially its related DUB modules, with which it shares the ENY2 subunit.

Depletion of the ENY2 alone surprisingly not only does not recapitulate GANP/TREX-2 effect on homologous recombination, but it has the opposite effect by stimulating HR efficiency. We ascribed this effect to the DUB function(s), because depletion of ATXN7L3 also increased HR efficiency to the same extent (Fig. 6a;d). Monoubiquitination of H2B, by RNF20/40 E3 ubiquitin ligase, happens in response to DNA damage in human cells and is required for recruitment of HR factors [28, 29]. Thus, our results are in good accordance with the data showing that depletion of RNF20/40 induces a decrease in HR efficiency and resection [28, 29], resulting in the opposite phenotype than the DUBm deficient cells.

We hypothesize that GANP/TREX-2 regulates homologous recombination through controlling the DUB module activity because we were able to rescue both H2Bub1 levels and RAD51 recruitment at the ISceI-induced break in GANP deficient cells by further abolishing the

DUBm functions through additional ENY2, or ATXN7L3, depletion (Fig. 7). Importantly, in spite of the fact that the SAGA DUBm is thought to be the major H2Bub1 DUB, depletion of the DUBm adaptors, ENY2 and/or ATXN7L3, would eliminate the activity of all the USP22-related DUBm-s (independently of the cellular expression levels of the three related USPs). Thus, ENY2 and/or ATXN7L3 depletion will affect all H2Bub1 deubiquitination activities[34]. Nevertheless, our results confirm that H2B/H2Bub1 balance is important for HR mediated repair and show that H2B/H2Bub1 balance is controlled by a regulated interplay between TREX-2 and SAGA DUB subunits ENY2 and ATXN7L3 (Fig. 8). How the H2Bub1 mark regulates repair is not clear. It was speculated that the HR impairment in RNF20 depleted cells was dependent on a defect in chromatin relaxation ascribed to the H2Bub1-dependent methylation of H3K4 [29]. However, in a second study H3K4 methylation was maintained unaltered in RNF20 KD, and accumulation of H3K4me was not observed at damaged sites [28]. Although a defect in chromatin relaxation was speculated in both studies, it cannot be excluded that H2Bub1 mark would be necessary for the direct recruitment of specific DNA repair factors to break sites.

In yeast, physical interactions between TREX-2 and SAGA have been described [30, 64], nevertheless such interactions have not been detected in *Drosophila* [65] and mammalian cells [17]. It is however conceivable that in mammalian cells TREX-2 and SAGA DUB also interact in a more dynamic and less stable manner that could be more difficult to detect in different metazoan cell extracts. It seems that the interaction between TREX-2 and the DUB module(s) can fine-tune the DUB activity(ies). It has been shown that the DUB module of SAGA exists in metazoan cells without incorporating in SAGA [66, 67] and that additional related DUBm-s exist, all able to deubiquitinate H2Bub1 [34]. Moreover, the SAGA DUB module was suggested to also have SAGA-independent functions [66]. Thus, the regulatory interplay between TREX-2 and the different DUB modules may not necessarily involve the SAGA complex. The fact that the NPC-associated TREX-2 complex regulates the activity of the nucleoplasmic DUB module(s), might also suggest that a nucleoplasmic pool of TREX-2 exists. Nevertheless, future investigations will be

needed to understand the exact mechanism through which TREX-2 can control the DUB activity(ies) and consequently DNA repair.

A similar regulatory interplay has been already described showing that cytoplasmic ATXN7L3B, which is encoded by a pseudogene that arose from the retro-transposition of *ATXN7L3*, may regulate the nuclear function of SAGA DUB module and H2Bub1 levels through competition for ENY2 binding [35]. Thus, it seems that the different ENY2-containing complexes, TREX-2, the SAGA DUB, the different SAGA-independent DUB modules and the cytoplasmic ATXN7L3B/ENY2 association, may all have important regulatory functions to achieve well balanced H2B/H2Bub1 levels that are required for efficient DNA repair and homologous recombination. These different complexes may compete with each other for ENY2 and thus depending on the availability of ENY2 for TREX-2 or the DUB modules, regulate H2Bub1 levels. How exactly neo-synthesized ENY2 is distributed in the different complexes by distinct assembly pathways need to be further investigated.

Materials and Methods:

Cell lines, NCS treatment and transfections

HeLa and U2OS cells were cultured in DMEM 1g/l. U2OS 19 ptight 13 GFP-LacI [55] were cultured as previously described [46, 55]. For NCS treatments cells were treated with 50 ng/ml (for immunofluorescence) or 150-250 ng/ml (for western blot analysis) of NCS (N9162, Sigma Aldrich) for 15 minutes. Medium was refreshed and cells were allowed to repair for indicated time points, before fixation or protein extraction. All indicated siRNAs were transfected in all cell lines using Lipofectamine 2000 (Lipofectamine® 2000 Thermo Fisher Scientific) following manufacturer's instructions. For High throughput screening two different siRNAs for GANP, (individually and pool of the two) and three different siRNAs fror ENY2 (individually and pool of the three) were used. For all the other experiments siGANP2 was used (referred as siGANP) and siENY2_2 was used (referred as siENY2). For siRNAs sequences and references see siRNA list.

Clonogenic survival assay

HeLa cells were transfected with indicated siRNAs and 48 h after transfection, cells were counted and seeded in triplicates in 6-well plates (500 cells per well). The day after, cells were treated with indicated concentration of phleomycin (Sigma, St Louis, MO, USA) or with camptothecin (C9911, Euromedex) for one hour. Medium was refreshed and cells were then cultured for 10 days. Colonies were stained with 0.1% crystal violet and counted using ImageJ.

DR-GFP assays

For HR efficiencies, U2OS DR-GFP Cherry-IsceI-GR cells [68] were transfected with the indicated siRNA and 24 h later Triamcinolone acetonide (TA) was added to allow translocation of Cherry-IsceI-GR into the nucleus for 48 hours. For NHEJ efficiency GCV6 cell line [52] bearing the GFP-based substrates was used. Cells were first transfected with the indicated siRNAs and 48 h after, they were transfected with HA-I-SceI expression vector

(pCBASce) using jetPei (Polyplus) following manufacturer's instructions. GFP intensities were measured by FACS and analysed using FlowJo software (TreeStar).

Immunofluorescence

Genome wide recruitment of RAD51 and 53BP1 was assessed in HeLa cells plated in 24 well plates on round cover glasses (VWR®). Recruitment of DNA repair factors at a single IsceI cut site was assessed in U2OS 19 ptight 13 GFP-LacI cells. Immunofluorescence was performed equally in both cell lines. At the decided time point cells were washed carefully with PBS 1X. Cells stained for RAD51 were treated with pre-extraction buffer (0.5% triton X; 50 mM HEPES pH 7; 150 mM NaCl; 10 mM EGTA; 2 mM $MgCl_2$) for 10 seconds before fixation with Paraformaldehyde (PFA) 4% for 10 minutes at RT. Cells stained for other factors were fixed with PFA immediately after washing. After fixation cells were washed with PBS 1X and permeabilized with sterile 0.3% Triton X in PBS for 10 minutes at RT, and blocked with sterile 5% BSA for 1 hour. Primary antibodies were added for 1 hour, cells were washed and stained with secondary fluorescent antibodies. For primary antibodies see Antibodies list. All the fluorescent secondary antibodies were Alexa Fluor Antibodies from Invitrogen. DAPI (Sigma Aldrich D9542) was added at 0.3 mM final concentration for 2 minutes and coverslips were mounted using ProLong® Gold Antifade Mountant (Life Technologies, P36934). Images were acquired using the fluorescence microscope Leica DM 4000 B or Confocal microscope Leica TCS SP5 Inverted. Images were analysed using Image J. For co-localization analyses of DNA repair factors with the LacO array co-localization was counted at the fluorescence microscope.

Cellular extracts and Western blot

Total protein lysate was obtained by adding cold RIPA buffer (Cold Spring Harbor Protocols) on the cell pellet. Histone proteins extract was obtained by adding acidic buffer (10 mM HEPES pH. 7.9; 1.5 mM $MgCl_2$; 10 mM KCl; 0.5 mM DTT; 0.2 M HCl) on cell pellet. 1X Proteinase Inhibitor Cocktail (PIC, cOmplete™, Mini EDTA free, Proteinase Inhibitor Cocktail

11836170001 Roche), Phosphatase Inhibitor Cocktail (PhosSTOP™ PHOSS-RO Roche) and 10 mM N-Ethylmaleimide (NEM E3876 Sigma Aldrich) were added in all buffers. Samples were run on 4–20% Precast gels (Mini-PROTEAN® TGX Stain-Free™ Protein Gels, Biorad) and membranes blotted with indicated antibodies. Western Blots were revealed using peroxidase conjugated secondary antibodies from Jackson Immunoresearch Laboratories. Images were acquired using Chemidoc™ Touch Imaging system (Biorad). Protein levels were quantified by Image J.

Cell cycle analysis

For propidium iodide stainining, after siRNAs treatement with the indicated siRNAs, U2OS cells were fixed in 70% EtOH overnight at −20°C, treated with RNase A (100 μg/ml) and stained with propidium iodide (40 μg/ml). For S-phase analysis, after siRNAs treatment with the indicated siRNAs, HeLa cells were collected and stained with EdU using Click-It kit (C10632, Life technologies) following manufacturer's instructions. In both cases the acquisition was performed on a FACSCalibur (Becton-Dickinson) Results were analysed using FlowJo software (TreeStar).

Comet assay

For the comet assay HeLa cells were treated with siRNAs for 48 hours before collection. Neutral COMET assay was performed using CometAssay® Kit Trevigen (4205-050-K) following manufacturer's instructions. Cells were analysed using OpenCOMET plug-in on ImageJ.

High throughput screening

Cells were treated with specific siRNA in 96 well plates, in three technical replicates (XRCC4 and SET siRNAs were used as positive controls). After 72 hours cells were treated with 50 ng/ml of NCS for 15 minutes. Medium was refreshed and cells were allowed to repair for 2, 8 and 16 hours. Cells were fixed and stained with DAPI, γH2AX and 53BP1 specific antibodies. Image acquisition was done using Cell insight and 20X objective. γH2AX intensity and 53BP1 foci number were analyzed with HCS studio. For γH2AX intensity distribution among

inner nuclear environment and nuclear periphery a mask of 2.5 μm (6 pixels) on the DAPI staining was applied to define the nuclear periphery. For each cell the intensity of the γH2AX signal in the periphery and in the whole nucleus was measured. The peripheral signal per each cell is represented by the ratio between the two values. The inner signal per each cell is represented by the ratio between the inner signal and the total signal.

RNA extracts and qPCR

RNA was extracted using NucleoSpin® RNA Kit (Macherey-Nagel) and cDNA was obtained with Superscript™ II Reverse Transcriptase (Invitrogen) following manufacturer's instructions. qPCR was performed using LightCycler® 480 SYBR Green I Master (Roche) following manufacturer's instructions. For primer sequence see primers' list. All the primers were purchased from Sigma Aldrich.

Antibodies

Antibody	Reference	Working dilution
Anti-γH2AX	Abcam (ab22551)	Western Blot and IF (1:1000)
Anti-GANP	Abcam (113295)	Western Blot 1:500
Anti 53BP1	Novus Biologicals (NB100-304)	IF (1:1000)
Anti-Phospho RPA32 (S4/8)	Bethyl (A300-245A)	IF (1:500)
Anti-Phospho RPA 32 (S33)	Bethyl (A300-246A)	IF (1:1000)
Anti-RAD51	Calbiochem (PC130)	IF (1:500) Western Blot (1:1000)
Anti-ATM pS1981	Rockland (200-301-400)	Western Blot (1:2000)
Anti-ENY2	Santa Cruz (sc-87712)	Western Blot (1:200)
Anti-γTUBULIN	Sigma (T6557)	Western Blot (1:1000)
Anti-phospho KAP1	Bethyl (A300-767A)	Western Blot (1:500)
Anti-GAPDH	Merck Millipore (MAB374)	Western Blot (1:1000)
Anti-BRCA1	Santa Cruz (C-20 SC642)	IF (1:100)
Anti-CtIP	Bethyl (A300-488A)	IF (1:100)
Anti-H2Bub1	Cell signalling (CS55465)	Western Blot (1:10000)
Anti-H2B	"in house" (H2-2A4) [69]	Western Blot (1:10000)
Anti-TBP	"in house" (mAb 3G3) [70]	Western Blot (1:1000)

Anti-αTUBULIN	Sidma (T9026)	Western Blot (1:1000)

siRNAs

siRNA	Reference
siCTRL	(siGANPc) [18]
siGANP1	[18]
siGANP2	[18]
siSCR	(GUUAACGAUAAUUAGAUAA) purchased from Sigma Aldrich
siENY2_1	Ambion (s32447)
siENY2_2	Ambion (s32449)
siENY2_3	Ambion (s326899)
siXRCC4	Dharmacon, siGenome, Smart Pool (M-004494-02-0020)
siSET	Dharmacon, on-TARGETplus (L-019586-00-0005)
siNTarg	Dharmacon, ON-TARGET plus, (D-001810-10-20)
siRAD51	Ambion (s11735)
siATXN7L3	Thermo scientific (271960)

Primers

Primer	Sequence
GANP_fw	CACGAGCCAGCAGCAGAAGTTC
GANP_rev	CATCCTGTATCGTCCGACCA
ENY2_fw	GGAGAAAGAGAACGCCTCAAA
ENY2_rev	AGTGATTTCAGCCACCAAGTCA
ATXN7L3_fw	CTGGGAATGGGTCGGAACAG
ATXN7L3_rev	CCGAGCCATAGGACCAGTCG
GAPDH_fw	TCGACAGTCAGCCGCATCTTCTTT
GAPDH_rev	ACCAAATCCGTTGACTCCGACCTT

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Author's contributions

FE performed most of the experiments. AMR performed the high throughput screening experiments. MS contributed to the execution of molecular lab work. FE, ES and LT designed the study, analysed data and wrote the paper. All authors contributed to text and figure panels to the manuscript. All authors gave final approval for publication.

References

1. Barnes, D.E. and T. Lindahl, *Repair and genetic consequences of endogenous DNA base damage in mammalian cells.* Annu Rev Genet, 2004. **38**: p. 445-76.
2. Lindahl, T. and D.E. Barnes, *Repair of endogenous DNA damage.* Cold Spring Harb Symp Quant Biol, 2000. **65**: p. 127-33.
3. Hoeijmakers, J.H., *DNA damage, aging, and cancer.* N Engl J Med, 2009. **361**(15): p. 1475-85.
4. Pfeiffer, P., W. Goedecke, and G. Obe, *Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations.* Mutagenesis, 2000. **15**(4): p. 289-302.
5. Iliakis, G., et al., *Mechanisms of DNA double strand break repair and chromosome aberration formation.* Cytogenet Genome Res, 2004. **104**(1-4): p. 14-20.
6. Dudley, D.D., et al., *Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences.* Adv Immunol, 2005. **86**: p. 43-112.
7. Rogakou, E.P., et al., *DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139.* J Biol Chem, 1998. **273**(10): p. 5858-68.
8. Orthwein, A., et al., *A mechanism for the suppression of homologous recombination in G1 cells.* Nature, 2015. **528**(7582): p. 422-6.
9. Liu, T. and J. Huang, *DNA End Resection: Facts and Mechanisms.* Genomics Proteomics Bioinformatics, 2016. **14**(3): p. 126-130.
10. Falck, J., et al., *CDK targeting of NBS1 promotes DNA-end resection, replication restart and homologous recombination.* EMBO Rep, 2012. **13**(6): p. 561-8.
11. Huertas, P., et al., *CDK targets Sae2 to control DNA-end resection and homologous recombination.* Nature, 2008. **455**(7213): p. 689-92.
12. Wang, H., et al., *The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair.* PLoS Genet, 2013. **9**(2): p. e1003277.
13. Faza, M.B., et al., *Sem1 is a functional component of the nuclear pore complex-associated messenger RNA export machinery.* J Cell Biol, 2009. **184**(6): p. 833-46.
14. Fischer, T., et al., *Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery.* Nat Cell Biol, 2004. **6**(9): p. 840-8.
15. Fischer, T., et al., *The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores.* EMBO J, 2002. **21**(21): p. 5843-52.
16. Kohler, A. and E. Hurt, *Exporting RNA from the nucleus to the cytoplasm.* Nat Rev Mol Cell Biol, 2007. **8**(10): p. 761-73.
17. Umlauf, D., et al., *The human TREX-2 complex is stably associated with the nuclear pore basket.* J Cell Sci, 2013. **126**(Pt 12): p. 2656-67.
18. Wickramasinghe, V.O., et al., *mRNA export from mammalian cell nuclei is dependent on GANP.* Curr Biol, 2010. **20**(1): p. 25-31.
19. Wickramasinghe, V.O., M. Stewart, and R.A. Laskey, *GANP enhances the efficiency of mRNA nuclear export in mammalian cells.* Nucleus, 2010. **1**(5): p. 393-6.
20. Koutelou, E., C.L. Hirsch, and S.Y. Dent, *Multiple faces of the SAGA complex.* Curr Opin Cell Biol, 2010. **22**(3): p. 374-82.
21. Grant, P.A., et al., *Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex.* Genes Dev, 1997. **11**(13): p. 1640-50.
22. Gavin, A.C., et al., *Functional organization of the yeast proteome by systematic analysis of protein complexes.* Nature, 2002. **415**(6868): p. 141-7.

23. Henry, K.W., et al., *Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8.* Genes Dev, 2003. **17**(21): p. 2648-63.
24. Sanders, S.L., et al., *Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry.* Mol Cell Biol, 2002. **22**(13): p. 4723-38.
25. Bonnet, J., et al., *The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription.* Genes Dev, 2014. **28**(18): p. 1999-2012.
26. Baptista, T., et al., *SAGA Is a General Cofactor for RNA Polymerase II Transcription.* Mol Cell, 2017.
27. Zhu, B., et al., *Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation.* Mol Cell, 2005. **20**(4): p. 601-11.
28. Moyal, L., et al., *Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks.* Mol Cell, 2011. **41**(5): p. 529-42.
29. Nakamura, K., et al., *Regulation of homologous recombination by RNF20-dependent H2B ubiquitination.* Mol Cell, 2011. **41**(5): p. 515-28.
30. Rodriguez-Navarro, S., et al., *Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery.* Cell, 2004. **116**(1): p. 75-86.
31. Jani, D., et al., *Functional and structural characterization of the mammalian TREX-2 complex that links transcription with nuclear messenger RNA export.* Nucleic Acids Res, 2012. **40**(10): p. 4562-73.
32. Lang, G., et al., *The tightly controlled deubiquitination activity of the human SAGA complex differentially modifies distinct gene regulatory elements.* Mol Cell Biol, 2011. **31**(18): p. 3734-44.
33. Zhang, X.Y., et al., *The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression.* Mol Cell, 2008. **29**(1): p. 102-11.
34. Atanassov, B.S., et al., *ATXN7L3 and ENY2 Coordinate Activity of Multiple H2B Deubiquitinases Important for Cellular Proliferation and Tumor Growth.* Mol Cell, 2016. **62**(4): p. 558-71.
35. Li, W., et al., *Cytoplasmic ATXN7L3B Interferes with Nuclear Functions of the SAGA Deubiquitinase Module.* Mol Cell Biol, 2016. **36**(22): p. 2855-2866.
36. Blobel, G., *Gene gating: a hypothesis.* Proc Natl Acad Sci U S A, 1985. **82**(24): p. 8527-9.
37. Cabal, G.G., et al., *SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope.* Nature, 2006. **441**(7094): p. 770-3.
38. Chekanova, J.A., et al., *Sus1, Sac3, and Thp1 mediate post-transcriptional tethering of active genes to the nuclear rim as well as to non-nascent mRNP.* RNA, 2008. **14**(1): p. 66-77.
39. Garcia-Oliver, E., V. Garcia-Molinero, and S. Rodriguez-Navarro, *mRNA export and gene expression: the SAGA-TREX-2 connection.* Biochim Biophys Acta, 2012. **1819**(6): p. 555-65.
40. Pascual-Garcia, P., et al., *Sus1 is recruited to coding regions and functions during transcription elongation in association with SAGA and TREX2.* Genes Dev, 2008. **22**(20): p. 2811-22.
41. Gallardo, M. and A. Aguilera, *A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination.* Genetics, 2001. **157**(1): p. 79-89.

42. Gallardo, M., et al., *Nab2p and the Thp1p-Sac3p complex functionally interact at the interface between transcription and mRNA metabolism.* J Biol Chem, 2003. **278**(26): p. 24225-32.
43. Gonzalez-Aguilera, C., et al., *The THP1-SAC3-SUS1-CDC31 complex works in transcription elongation-mRNA export preventing RNA-mediated genome instability.* Mol Biol Cell, 2008. **19**(10): p. 4310-8.
44. Rondon, A.G., et al., *Molecular evidence for a positive role of Spt4 in transcription elongation.* EMBO J, 2003. **22**(3): p. 612-20.
45. Duheron, V., et al., *Localisation of Nup153 and SENP1 to nuclear pore complexes is required for 53BP1-mediated DNA double-strand break repair.* J Cell Sci, 2017. **130**(14): p. 2306-2316.
46. Lemaitre, C., et al., *The nucleoporin 153, a novel factor in double-strand break repair and DNA damage response.* Oncogene, 2012. **31**(45): p. 4803-9.
47. Mackay, D.R., et al., *Nup153 and Nup50 promote recruitment of 53BP1 to DNA repair foci by antagonizing BRCA1-dependent events.* J Cell Sci, 2017.
48. Kalousi, A., et al., *The nuclear oncogene SET controls DNA repair by KAP1 and HP1 retention to chromatin.* Cell Rep, 2015. **11**(1): p. 149-63.
49. Li, Z., et al., *The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination.* Cell, 1995. **83**(7): p. 1079-89.
50. Mimori, T. and J.A. Hardin, *Mechanism of interaction between Ku protein and DNA.* J Biol Chem, 1986. **261**(22): p. 10375-9.
51. Weinstock, D.M., et al., *Assaying double-strand break repair pathway choice in mammalian cells using a targeted endonuclease or the RAG recombinase.* Methods Enzymol, 2006. **409**: p. 524-40.
52. Rass, E., et al., *Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells.* Nat Struct Mol Biol, 2009. **16**(8): p. 819-24.
53. Sung, P., et al., *Rad51 recombinase and recombination mediators.* J Biol Chem, 2003. **278**(44): p. 42729-32.
54. Liu, L.F., et al., *Mechanism of action of camptothecin.* Ann N Y Acad Sci, 2000. **922**: p. 1-10.
55. Lemaitre, C., et al., *Nuclear position dictates DNA repair pathway choice.* Genes Dev, 2014. **28**(22): p. 2450-63.
56. Daley, J.M., et al., *Biochemical mechanism of DSB end resection and its regulation.* DNA Repair (Amst), 2015. **32**: p. 66-74.
57. Yu, X., et al., *The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression.* J Biol Chem, 1998. **273**(39): p. 25388-92.
58. Yun, M.H. and K. Hiom, *CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle.* Nature, 2009. **459**(7245): p. 460-3.
59. Shiloh, Y., et al., *RNF20-RNF40: A ubiquitin-driven link between gene expression and the DNA damage response.* FEBS Lett, 2011. **585**(18): p. 2795-802.
60. Bhatia, V., et al., *BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2.* Nature, 2014. **511**(7509): p. 362-5.
61. Eid, M.M., et al., *GANP regulates the choice of DNA repair pathway by DNA-PKcs interaction in AID-dependent IgV region diversification.* J Immunol, 2014. **192**(12): p. 5529-39.
62. Yoshida, M., et al., *GANP suppresses DNA recombination, measured by direct-repeat beta-galactosidase gene construct, but does not suppress the type of recombination applying to immunoglobulin genes in mammalian cells.* Genes Cells, 2007. **12**(10): p. 1205-13.

63. Moudry, P., et al., *Nucleoporin NUP153 guards genome integrity by promoting nuclear import of 53BP1.* Cell Death Differ, 2012. **19**(5): p. 798-807.
64. Kohler, A., et al., *Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export.* Nat Cell Biol, 2008. **10**(6): p. 707-15.
65. Weake, V.M., et al., *Post-transcription initiation function of the ubiquitous SAGA complex in tissue-specific gene activation.* Genes Dev, 2011. **25**(14): p. 1499-509.
66. Li, X., et al., *Enzymatic modules of the SAGA chromatin-modifying complex play distinct roles in Drosophila gene expression and development.* Genes Dev, 2017. **31**(15): p. 1588-1600.
67. Nagy, Z., et al., *The human SPT20-containing SAGA complex plays a direct role in the regulation of endoplasmic reticulum stress-induced genes.* Mol Cell Biol, 2009. **29**(6): p. 1649-60.
68. Shahar, O.D., et al., *Live imaging of induced and controlled DNA double-strand break formation reveals extremely low repair by homologous recombination in human cells.* Oncogene, 2012. **31**(30): p. 3495-504.
69. Bonnet, J., et al., *The structural plasticity of SCA7 domains defines their differential nucleosome-binding properties.* EMBO Rep, 2010. **11**(8): p. 612-8.
70. Brou, C., et al., *Distinct TFIID complexes mediate the effect of different transcriptional activators.* EMBO J, 1993. **12**(2): p. 489-99.

Figure Legends

Fig. 1 Depletion of GANP affects DNA repair. (a) Clonogenic survival assay in HeLa cells depleted of GANP, and control cells, treated with increasing concentration of phleomycin. The graph represents the average of three independent experiments with three technical replicates each, where the number of colonies for each concentration was normalized to the respective untreated condition. Statistical significance was calculated using the Mann-Whitney test (ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$) **(b)** Western blot analyses of GANP and γH2Ax of RIPA extracts obtained from GANP-depleted cells and control cells. Numbers indicate γH2Ax levels quantified using ImageJ using γTubulin as loading control. **(c)** Graph represents the average of γH2Ax levels quantified using ImageJ upon three independent experiments. **(d)** DNA damage measured with the neutral comet assay of GANP-depleted cells and control cells. The number of breaks are represented in tail moment as the tail length normalized to the percentage of DNA in the tail. Graph represents the average of two independent experiments with $n \geq 100$ cells.

Fig. 2 Depletion of GANP/TREX-2 affects DNA repair without affecting the activation of the DNA Damage Response. (a-b) High throughput screening carried out to investigate the effect of GANP knockdown in DNA repair in HeLa cells. Graphs represent the percentage of positive cells for γH2Ax intensity **(a)** and 53BP1 foci **(b)** compared to the untreated sample (NT). The X-axis shows the corresponding siRNA used. Black and grey boxes represent the hours of recovery after NCS treatment at 50 ng/ml for 15 minutes or the untreated (NO DRUG). A positive cell is defined as a cell having γH2AX intensity, or number of 53BP1 foci, higher than an arbitrary threshold defined on the 5% of untreated (NT) cells. Graphs represent the average of two independent experiments, each of them performed in three technical replicates, with standard deviations. Per each condition $n > 1000$ cells. **(c)** Western blot analysis of total cell lysate obtained by RIPA extraction from cells treated with GANP siRNA and control cells. Hours represent the time of repair after NCS treatment. NT represents the untreated control.

Fig. 3 Depletion of GANP affects the efficiency of homologous recombination. (a) HR efficiency in GANP-depleted cells and control cells. The frequency of HR mediated repair is analyzed by flow cytometry as the percentage of GFP positive cells (see Methods). Graph represents the mean of three independent experiments with standard deviations. RAD51 siRNA was used as a positive control for reduction of HR efficiency. Statistical significance was calculated using the t-test (** $P \leq 0.01$; *** $P \leq 0.001$). **(b)** Clonogenic survival assay of HeLa cells depleted of GANP and control cells treated with increasing concentration of camptothecin. The graph represents the average of three independent experiments with three technical replicates each, where the number of colonies per each concentration was normalized to the respective untreated condition. Statistical significance was calculated using the Mann-Whitney test (ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$). **(c)** NHEJ efficiency in control cells and cells depleted of GANP. The frequency of NHEJ mediated repair was analyzed by flow cytometry as the percentage of GFP positive cells (see Methods). Graph represents the mean of four independent experiments with standard deviations.

Fig. 4 Resection is impaired in GANP-depleted cells (a-f) Graphs represent time course of the percentage of colocalization of resection factors with the LacO array after doxycline addition in U2OS 19 ptight 13 cells stably expressing GFP-LacI. Cells were depleted of GANP or treated with control siRNA 48 hours prior to doxycicline addition at a final concentration of 1 mg/ml. Values represent the merge of at least three independent experiments with $n \geq 50$ cells per condition. Statistical significance was calculated using t-test (ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

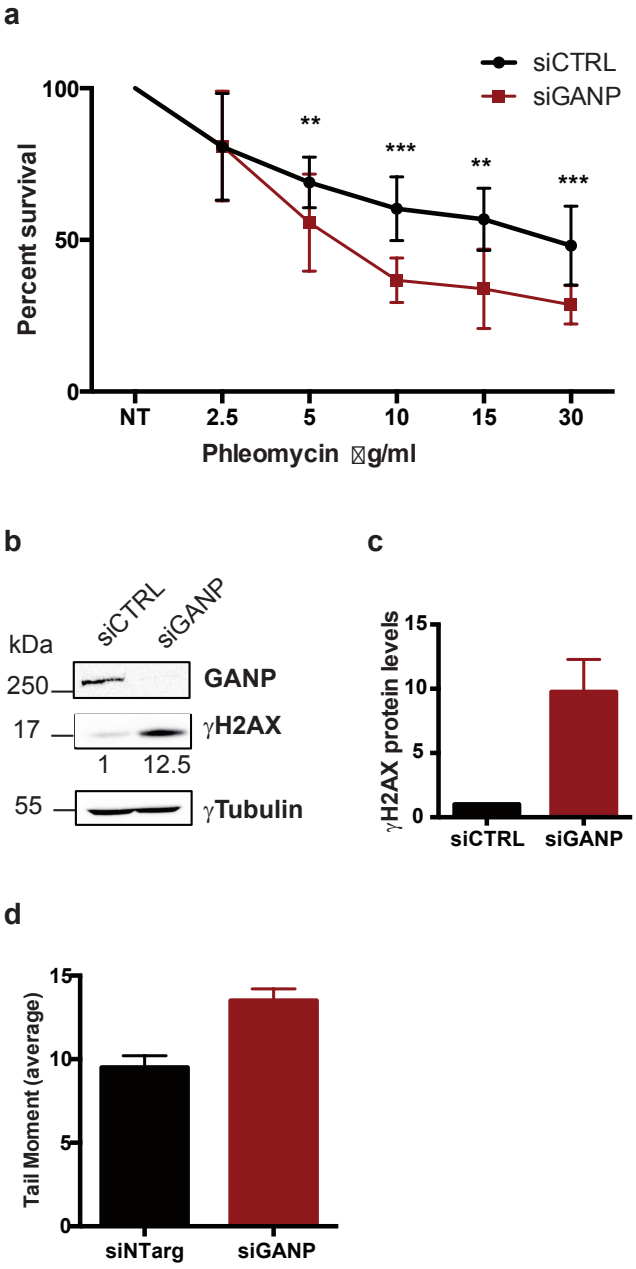
Fig. 5 RAD51 recruitment is impaired in GANP-depleted cells (a) Representative images of HeLa cells stained with DAPI (in blue) and RAD51 (in red). Hours represent the time of repair after NCS treatment for 15 minutes at 50 ng/ml. **(b)** Graphs represent the percentage of cells showing

more than 5 RAD51 foci of four independent experiments with standard deviations. For each experiments 10 pictures were acquired using confocal microscope, with more than 100 cells per condition. Statistical significance was calculated using the t-test (** $P \leq 0.01$).

Fig. 6 ENY2 and ATXN7L3 are required to avoid unscheduled homologous recombination. (a and d) Frequency of HR mediated repair in either ENY2 **(a)** or ATXN7L3 **(d)** depleted cells Analysis was performed as described in Fig. 3a. Graphs represent the average of three independent experiments with standard deviations Statistical significance was calculated using the t-test (* $P \leq 0.05$; ** $P \leq 0.01$). **(b)** Clonogenic survival assay of HeLa cells depleted of ENY2 and control cells treated with increasing concentration of camptothecin. The graph represents the average of three independent experiments with three technical replicates each, where the number of colonies per each concentration was normalized to the respective untreated condition. Statistical significance was calculated using the Mann-Whitney test (ns $P > 0.05$; * $P \leq 0.05$). The experiment was performed in parallel with the experiment in Fig. 3b thus the control is the same. **(c)** High throughput screening was carried out to investigate the effect of ENY2 knockdown in DNA repair in HeLa cells (for details refer to Fig. 2a).

Fig. 7 GANP/TREX-2 affects homologous recombination through H2Bub1. (a-d, g) Western blot assays of total cell lysate (RIPA) or histones extracts (ACIDIC) in cells treated with indicated siRNAs. Membranes were blotted with depicted antibodies. **(e-f)** Graphs represent time course of the percentage of colocalization of RAD51 with the LacO array (as described in Fig. 4) in siGANP conditions and co-depletion of GANP and ENY2 **(e)** or GANP and ATXN7L3 **(f)**. Values represent the merge of at least three independent experiments with $n \geq 50$ cells per condition. Statistical significance was calculated using t-test (ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$).

Fig. 8 Model In steady state conditions TREX-2 and SAGA DUB share ENY2 subunit and H2B/H2Bub1 balance is maintained. In GANP knockdown ENY2 is redistributed to the DUB and this contributes to increase removal of ubiquitin from H2B and consequently HR is impaired. In ENY2 knockdown the DUB module is destabilized and this contributes to increase H2Bub1 levels, and consequently unscheduled HR


a
siCTRL
siGANP
100
50
0
Percent survival
**
***
**
***
NT
2.5
5
10
15
30
Phleomycin g/ml
b
siCTRL
siGANP
kDa
250
GANP
17
γH2AX
1
12.5
55
γTubulin
c
γH2AX protein levels
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5
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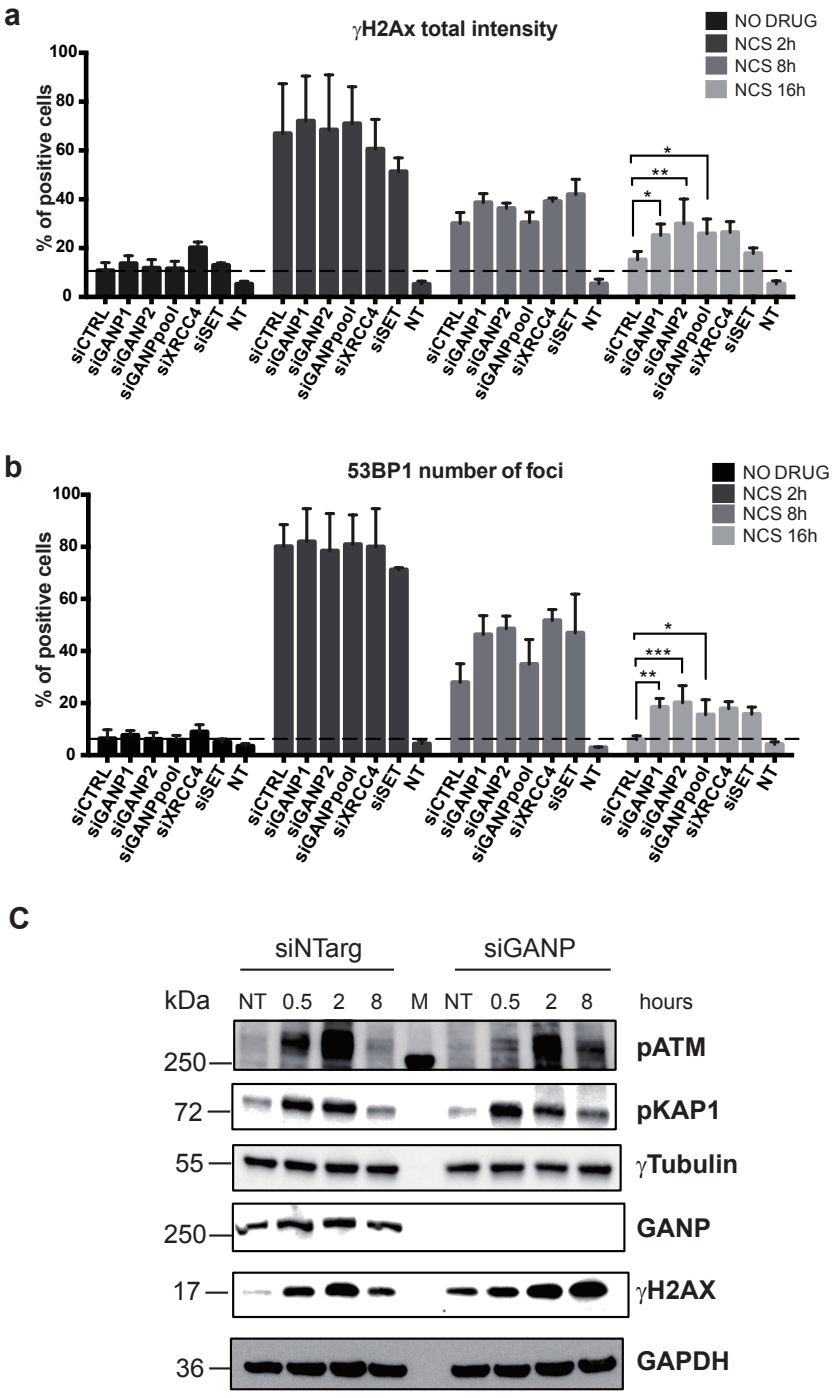
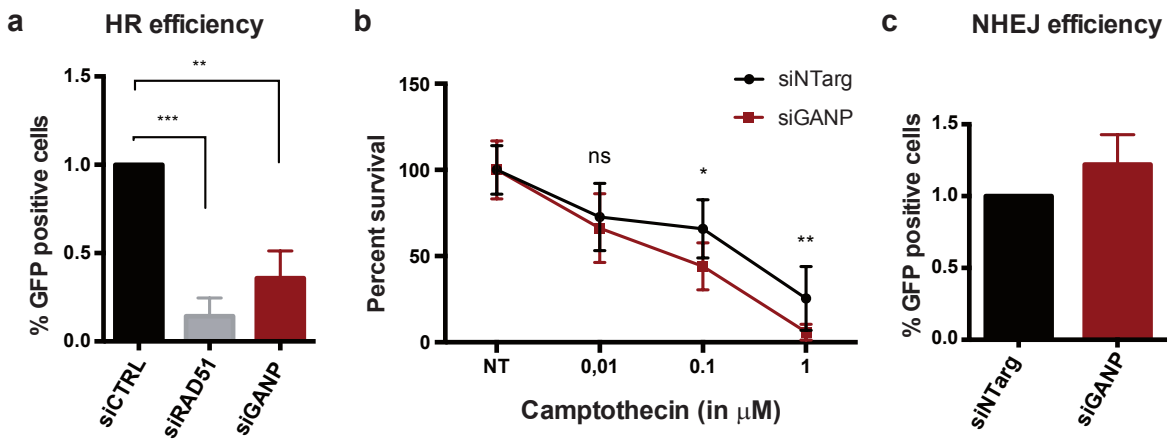
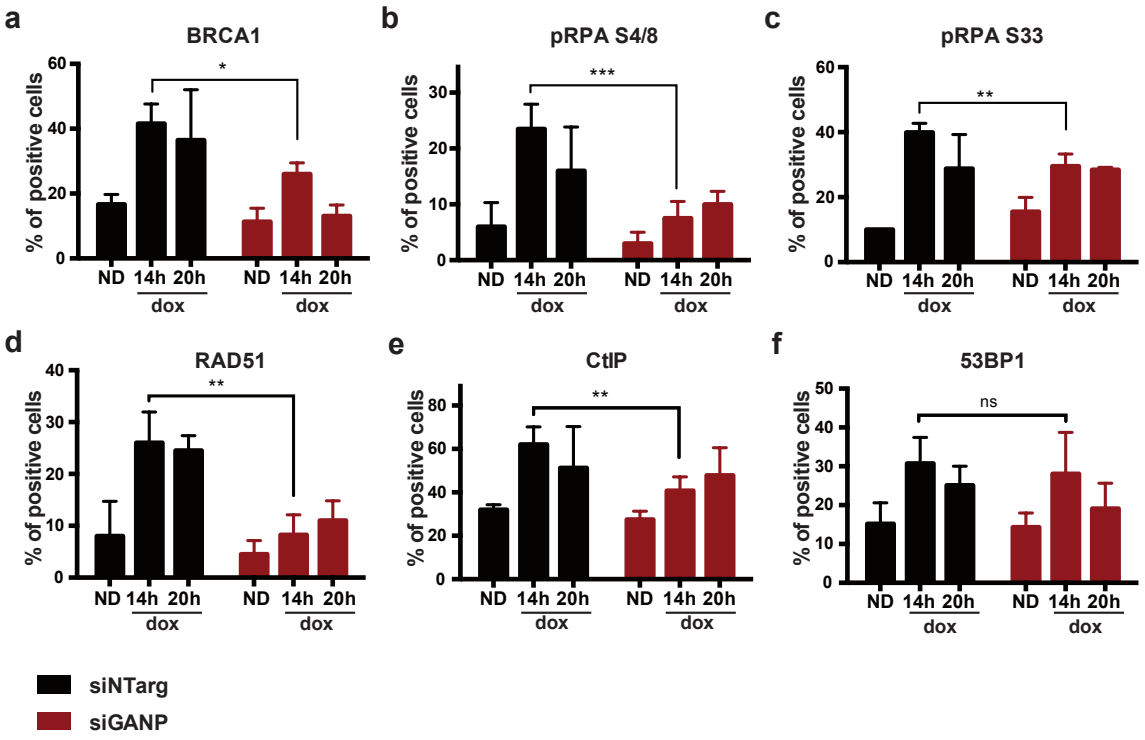
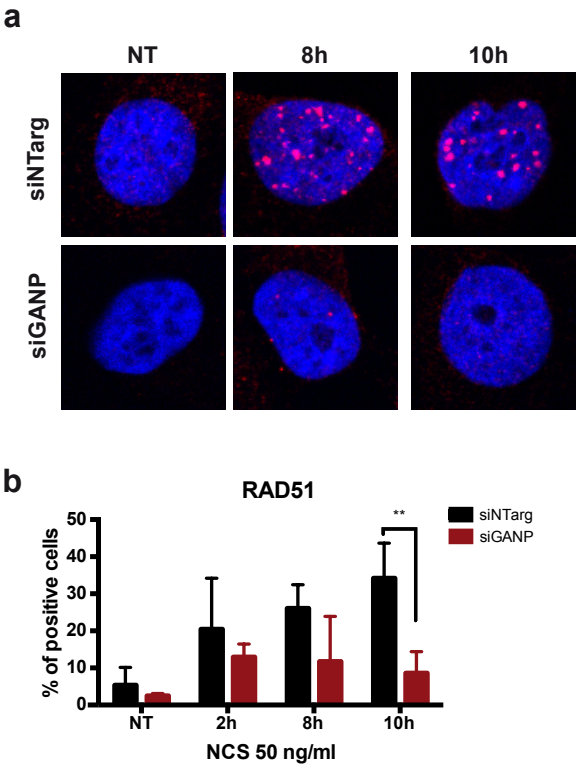
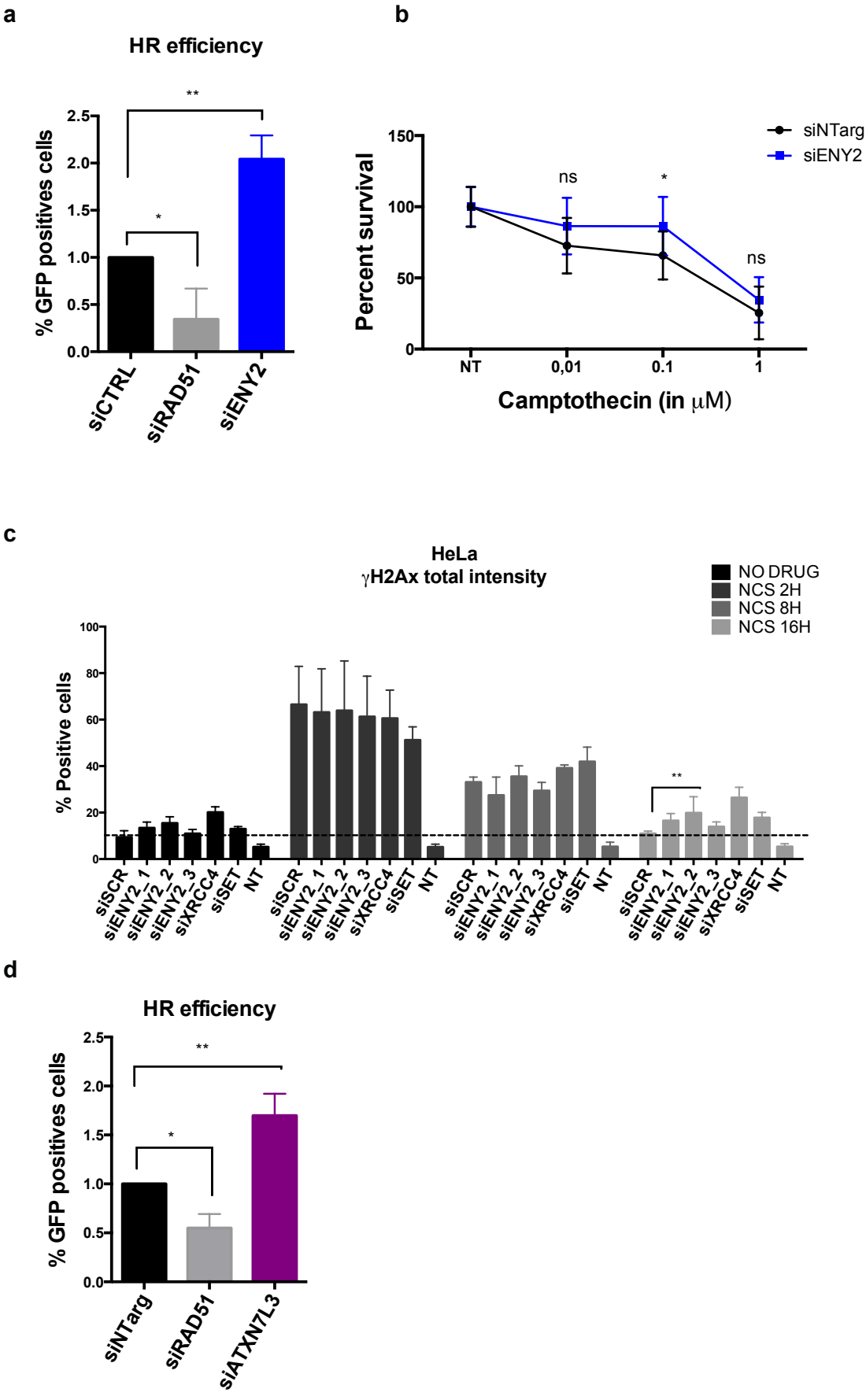
siGANP
d
Tail Moment (average)
15
10
5
0
siNTarg
siGANP

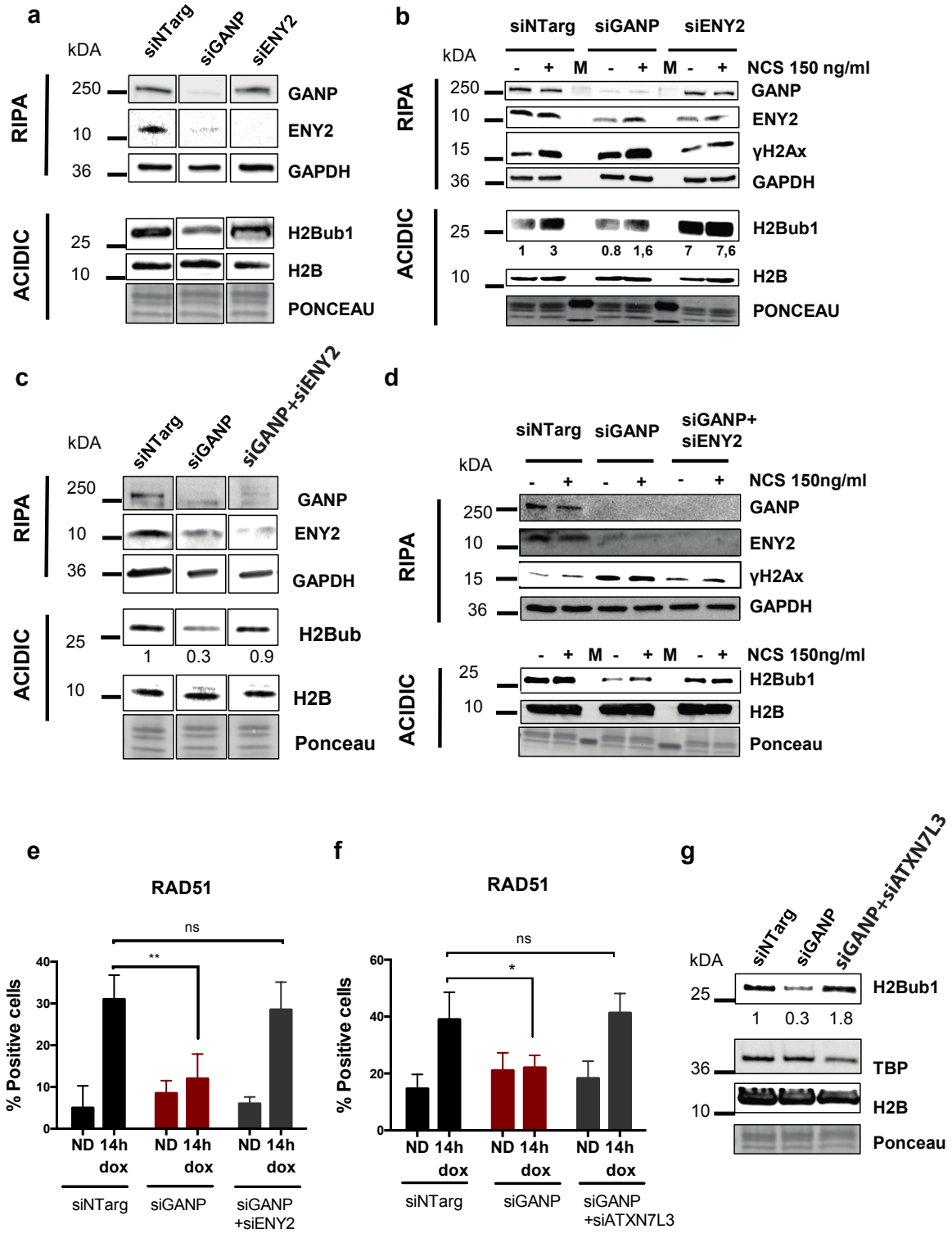

a
γH2Ax total intensity
NO DRUG
NCS 2h
NCS 8h
NCS 16h
% of positive cells
100
80
60
40
20
0
siCTRL
siGANP1
siGANP2
siGANPpool
siXRCC4
siSET
NT
b
53BP1 number of foci
NO DRUG
NCS 2h
NCS 8h
NCS 16h
% of positive cells
c
siNTarg
siGANP
kDa
NT 0.5 2 8 M NT 0.5 2 8
hours
250
pATM
72
pKAP1
55
γTubulin
250
GANP
17
γH2AX
36
GAPDH


a
HR efficiency
% GFP positive cells
1.5
1.0
0.5
0.0
**
***
siCTRL
siRAD51
siGANP
b
Percent survival
150
100
50
0
siNTarg
siGANP
ns
*
**
NT
0,01
0.1
1
Camptothecin (in μM)
c
NHEJ efficiency
% GFP positive cells
1.5
1.0
0.5
0.0
siNTarg
siGANP


a
BRCA1
% of positive cells
*
ND 14h 20h ND 14h 20h
dox dox
b
pRPA S4/8
% of positive cells
***
ND 14h 20h ND 14h 20h
dox dox
c
pRPA S33
% of positive cells
**
ND 14h 20h ND 14h 20h
dox dox
d
RAD51
% of positive cells
**
ND 14h 20h ND 14h 20h
dox dox
e
CtIP
% of positive cells
**
ND 14h 20h ND 14h 20h
dox dox
f
53BP1
% of positive cells
ns
ND 14h 20h ND 14h 20h
dox dox
siNTarg
siGANP


a
NT
8h
10h
siNTarg
siGANP
b
RAD51
siNTarg
siGANP
**
% of positive cells
50
40
30
20
10
0
NT
2h
8h
10h
NCS 50 ng/ml


a
HR efficiency
% GFP positives cells
siCTRL
siRAD51
siENY2
b
siNTarg
siENY2
Percent survival
ns
*
ns
NT
0,01
0.1
1
Camptothecin (in μM)
c
HeLa
γH2Ax total intensity
NO DRUG
NCS 2H
NCS 8H
NCS 16H
% Positive cells
siSCR
siENY2_1
siENY2_2
siENY2_3
siXRCC4
siSET
NT
d
HR efficiency
% GFP positives cells
siNTarg
siRAD51
siATXN7L3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kDA
siNTarg
siGANP
siENY2
RIPA
250
GANP
10
ENY2
36
GAPDH
ACIDIC
25
H2Bub1
10
H2B
PONCEAU
b
siNTarg
siGANP
siENY2
kDA
- + M - + M - +
NCS 150 ng/ml
RIPA
250
GANP
10
ENY2
15
γH2Ax
36
GAPDH
ACIDIC
25
H2Bub1
1 3 0.8 1,6 7 7,6
10
H2B
PONCEAU
c
kDA
siNTarg
siGANP
siGANP+siENY2
RIPA
250
GANP
10
ENY2
36
GAPDH
ACIDIC
25
H2Bub
1 0.3 0.9
10
H2B
Ponceau
d
siNTarg
siGANP
siGANP+
siENY2
kDA
- + - + - +
NCS 150ng/ml
RIPA
250
GANP
10
ENY2
15
γH2Ax
36
GAPDH
- + M - + M - +
NCS 150ng/ml
ACIDIC
25
H2Bub1
10
H2B
Ponceau
e
RAD51
ns
**
% Positive cells
40
30
20
10
0
ND 14h
dox
ND 14h
dox
ND 14h
dox
siNTarg
siGANP
siGANP
+siENY2
f
RAD51
ns
*
% Positive cells
60
40
20
0
ND 14h
dox
ND 14h
dox
ND 14h
dox
siNTarg
siGANP
siGANP
+siATXN7L3
g
kDA
siNTarg
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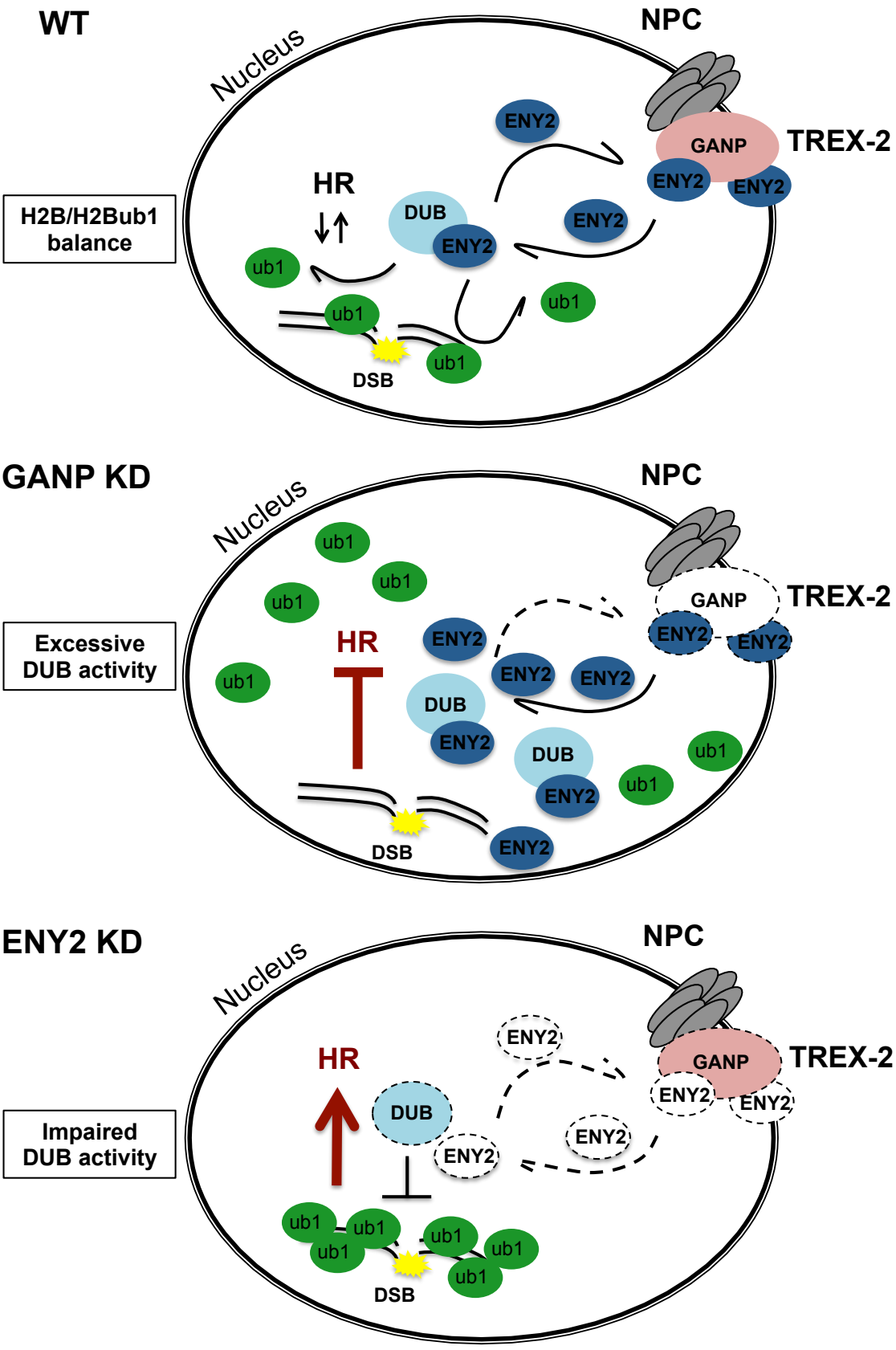
1 0.3 1.8
36
TBP
10
H2B
Ponceau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NPC
Nucleus
ENY2
GANP
TREX-2
ENY2
ENY2
HR
H2B/H2Bub1
balance
DUB
ENY2
ENY2
ub1
ub1
ub1
DSB
ub1
GANP KD
NPC
Nucleus
ub1
ub1
ub1
GANP
TREX-2
ENY2
ENY2
Excessive
DUB activity
HR
ENY2
ub1
ENY2
ENY2
DUB
ENY2
DUB
ub1
ENY2
ub1
DSB
ENY2
ENY2 KD
NPC
Nucleus
ENY2
HR
GANP
TREX-2
ENY2
ENY2
DUB
ENY2
ENY2
Impaired
DUB activity
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ub1
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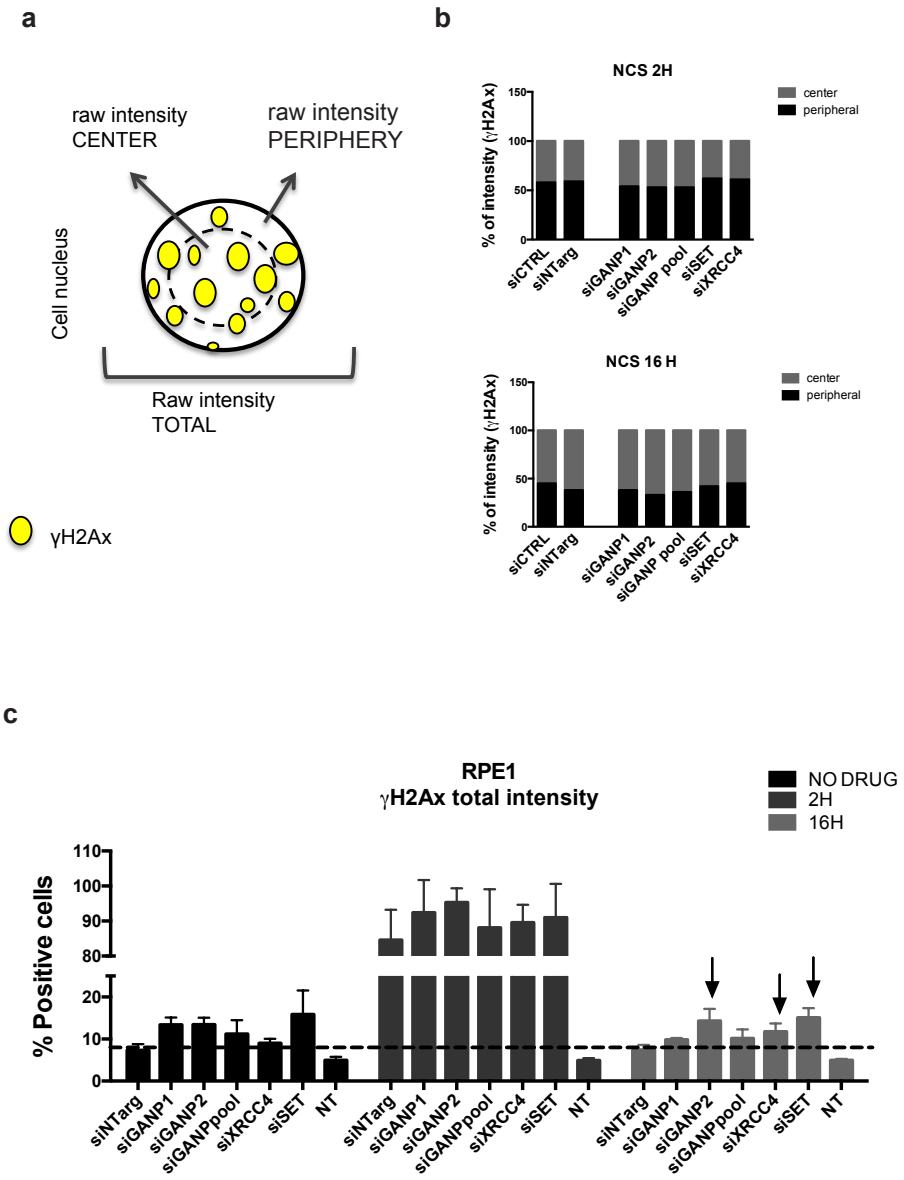
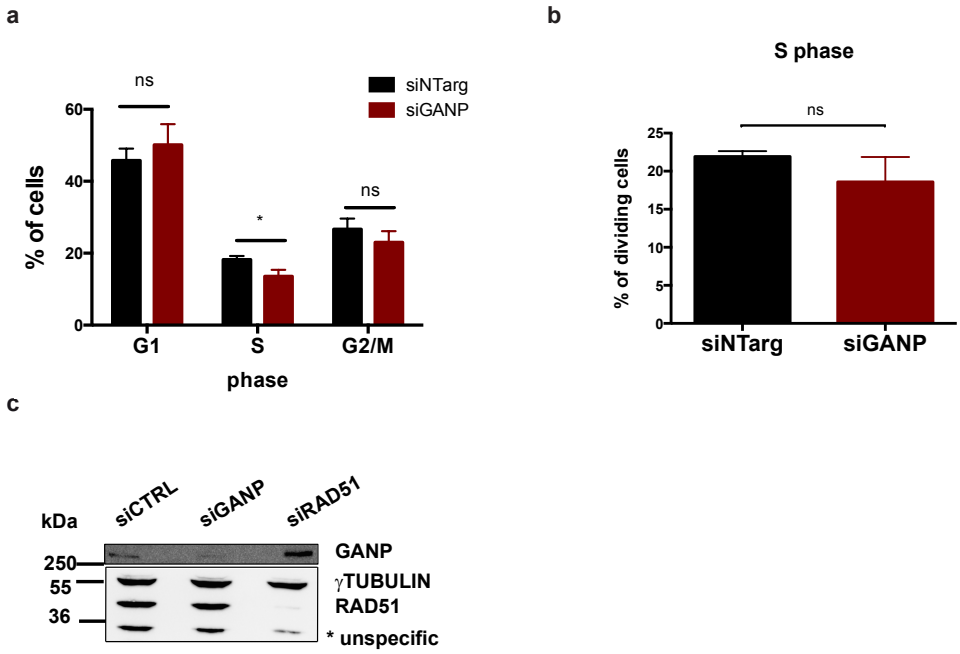
ub1
DSB

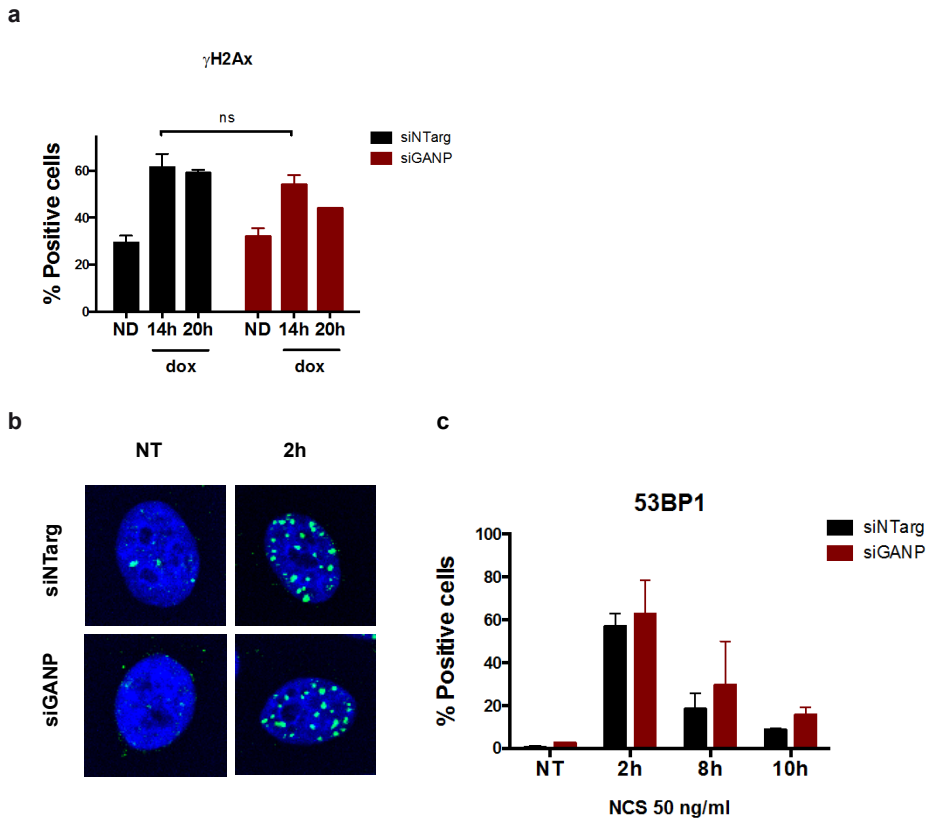
Supplementary Figures and Figure Legends



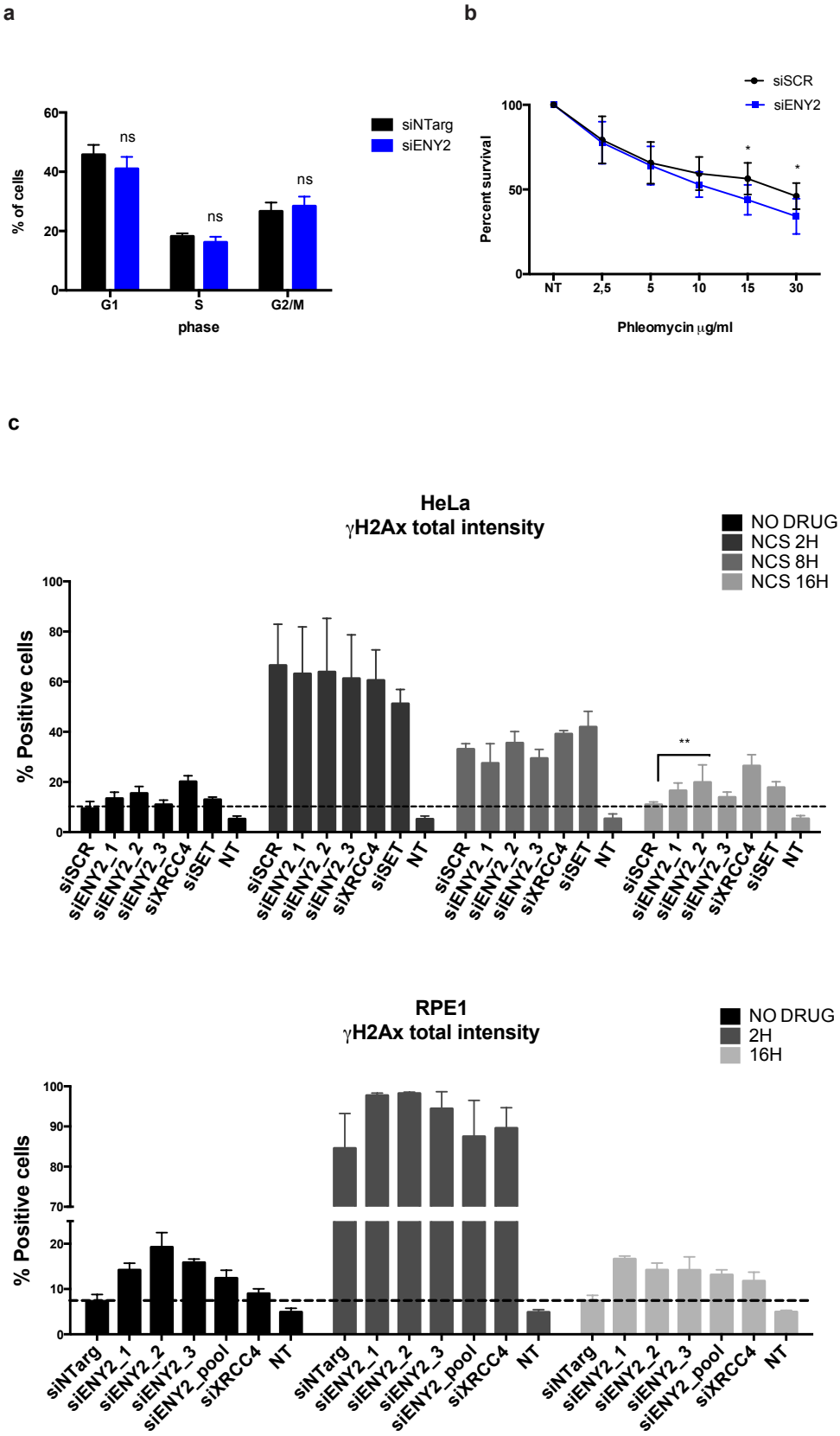
Supplementary Fig. 1 GANP affects global DNA repair. (a) Schematic representation of the cell nucleus after DNA damage induction with NCS and the applied masks to differentiate between peripheral and central nuclear environment. **(b)** Comparison of the percentage of intensity for γH2AX signal between peripheral and inner nuclear environment of the high throughput screening in Fig. 2a and b. n > 1000 cells **(c)** High throughput screening was performed to investigate the effect of GANP/TREX-2 knockdown in hTERT RPE1 cells. The control siRNA for RPE1 cells is represented by siNTarg. Per each condition n > 1000 cells (for details refer to Fig. 2a).



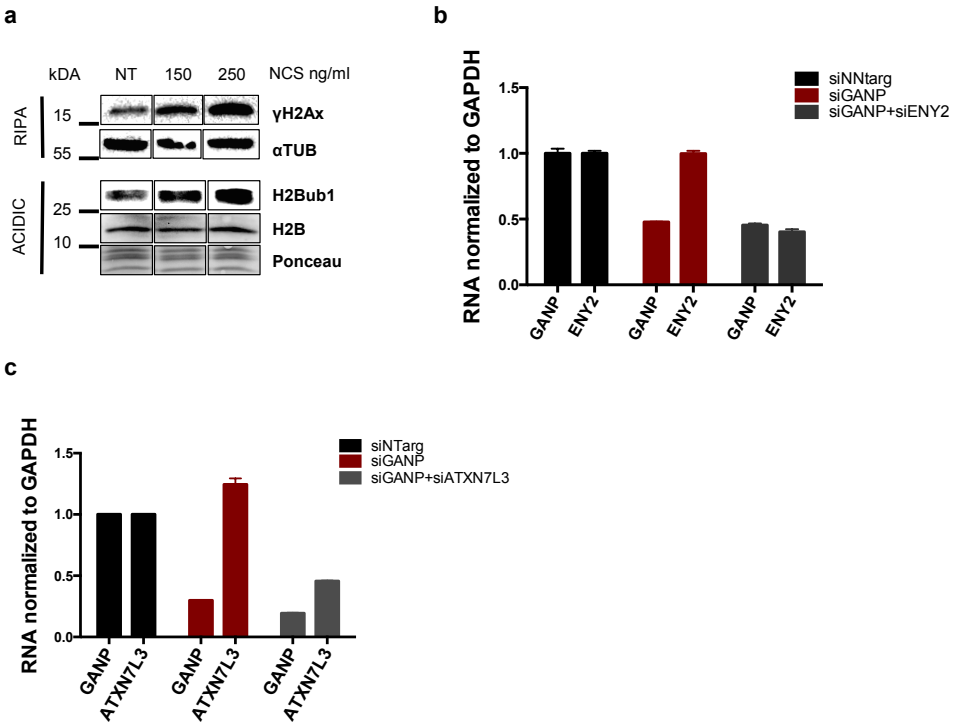
Supplementary Fig. 2 GANP depletion does not affect cell cycle progression and RAD51 protein levels. (a) Cell cycle analysis of U2OS cells treated with GANP siRNA and control siRNA for 48 hours prior to propidium iodide staining. Graph represents the percentage of cells in G1, S and G2/M phase of the cell cycle, based on DNA content **(b)** Analysis of HeLa cells in S phase after treatment with GANP siRNA and control siRNA for 48 hours. Graph represents the percentage of cells in S-phase using EdU staining. **(c)** Western blot analyses of total cell lysate obtained by RIPA extraction from cells treated with GANP siRNA, RAD51 siRNA and control siRNA for 48 hours. Membrane was stained with the indicated antibodies.



Supplementary Fig. 3 Efficient induction of breaks in GANP-depleted cells. (a) Graph represent time course of the percentage of co-localization of γH2AX with the LacO array after doxycline addition in U2OS 19 ptight 13 cells stably expressing GFP-LacI (for details see Fig. 4). Statistical significance was calculated using t-test (ns $P > 0.05$). **(b)** Representative images of HeLa cells stained with DAPI (in blue) and 53BP1 (in green). Hours represent the time of repair after treatment with NCS for 15 minutes and NT represents the untreated **(c)** Graph represents the percentage of cells showing more than 5 53BP1 foci of four independent experiments with standard deviations. For each experiment 10 pictures were acquired using confocal microscope, with $n > 100$ cells per condition.


a
b
c
% of cells
ns
ns
ns
G1
S
G2/M
phase
siNTarg
siENY2
Percent survival
siSCR
siENY2
NT
2,5
5
10
15
30
Phleomycin μg/ml
HeLa
γH2Ax total intensity
NO DRUG
NCS 2H
NCS 8H
NCS 16H
% Positive cells
siSCR
siENY2_1
siENY2_2
siENY2_3
siXRCC4
siSET
NT
RPE1
γH2Ax total intensity
NO DRUG
2H
16H
% Positive cells
siNTarg
siENY2_1
siENY2_2
siENY2_3
siENY2_pool
siXRCC4
NT

Supplementary Fig. 4 ENY2 depletion does not affect cell cycle progression and is required for efficient DSBs repair. (a) Cell cycle analysis of U2OS cells treated with ENY2 siRNA and control siRNA for 48 hours prior to propidium iodide staining. Graph represents the percentage of cells in G1, S and G2/M phase of the cell cycle, based on DNA content **(b)** Clonogenic survival assay of HeLa cells depleted of ENY2 and control cells treated with increasing concentration of phleomycin. The graph represents the average of three independent experiments with three technical replicates each, where the number of colonies per each concentration was normalized to the respective untreated (NT) condition. Statistical significance was calculated using the Mann-Whitney test (ns $P > 0.05$; * $P \leq 0.05$). **(c)** High throughput screening was carried out to investigate the effect of ENY2 knockdown in DNA repair in HeLa cells (top graph) and hTERT RPE1 cells (bottom graph). Control siRNAs are represented by a scramble (siSCR) in HeLa cells and siNTarg in RPE1 cells. Per each condition $n > 1000$ cells (For details refer to Fig. 2a).



Supplementary Fig. 5 Efficient double depletion of GANP/ENY2 and GANP/ATXN7L3. (a) Western blot analysis of total protein lysate (RIPA) and histone proteins (ACIDIC) of HeLa cells upon increasing concentration of NCS. Membranes were blotted with depicted antibodies. **(b-c)** Quantification of GANP, ENY2 and ATXN7L3 RNA levels by qPCR analysis. Graph represents the levels of the RNA normalized to GAPDH RNA levels. On the X-axis is depicted the analyzed RNA with specific primers. Colored boxes represent the different siRNA treatments.

3.1.1 DDR activation induces stabilization of ENY2

A characteristic of many proteins involved in the DDR is their recruitment and stabilization at the sites of breaks. *In vivo* studies of protein dynamics at the sites of damage include imaging of the fluorescent-tagged version of the protein, or biochemical approaches. Irradiation induces transcription of some genes expressing proteins involved in specific repair pathways, however many proteins are present in steady state levels and become simply activated after damage induction, by being post-translationally modified. As mentioned in the introduction ENY2 is small unstable protein of 11 kDA and its degradation is visible upon GANP KD as a result of protein destabilization upon loss of one of its main partners (Umlauf et al., 2013). Recent findings reported a new interaction between ENY2 and ATXN7L3B, and indicated that loss of ATXN7L3B by siRNA depletion also results in concomitant degradation of ENY2 (Li et al., 2016). Moreover additional DUBs, not incorporated in SAGA, but containing ENY2 and ATXN7L3 have been recently reported (Atanassov et al., 2016).

The above-mentioned results, together with our findings showing the importance of TREX-2 and SAGA DUB interplay in the DDR, suggest that ENY2 protein levels and distribution among different complexes play an important role in the DDR. For this reason we decided to investigate the effect of the activation of the DDR on ENY2 protein level. We conducted western blot analyses in asynchronous HeLa cells treated with NCS at a fixed concentration of 50 ng/ml for 15 minutes and then allowed to repair for 2 and 16 hours. Surprisingly ENY2 levels were increased after 2 hours of repair of an average of 3-fold in two independent experiments (Figure 19A and B), while GANP levels did not show any significant change (Figure 19B). To understand if our result was indeed a consequence of the damage induction, we conducted the same experiment treating the cells with increasing concentrations of NCS from 50 ng/ml to 200 ng/ml and we let cells repair for 2 hours after different treatments. Strikingly we observed a dose-dependent increase in ENY2 protein levels (Figure 19D and E). These results suggest that ENY2 levels are regulated in a damage-dependent manner. Noteworthy we detect a decrease in GANP protein level that was not visible at low NCS doses (Figure 19D).

In all the described DUB modules ATXN7L3 is the partner of ENY2. It has been previously shown that ATXN7L3 is degraded upon ENY2 depletion (Umlauf et al., 2013) thus we sought to determine if ATXN7L3 would show the same fluctuations upon damage induction. However we did not detect any significant change in ATXN7L3 protein levels (Figure 19D).

To understand if the observed changes in ENY2 protein levels were due to an induction of ENY2 at the transcription level we performed qPCR analysis. We did not detect any significant change in ENY2 and GANP mRNA levels at any time point after treatment with 50 ng/ml of NCS (Figure 19C), and we did not detect the same progressive increase in a concentration dependent manner (Figure 19F) thus we conclude that ENY2 undergoes a process of protein stabilization upon damage induction.

Figure 19

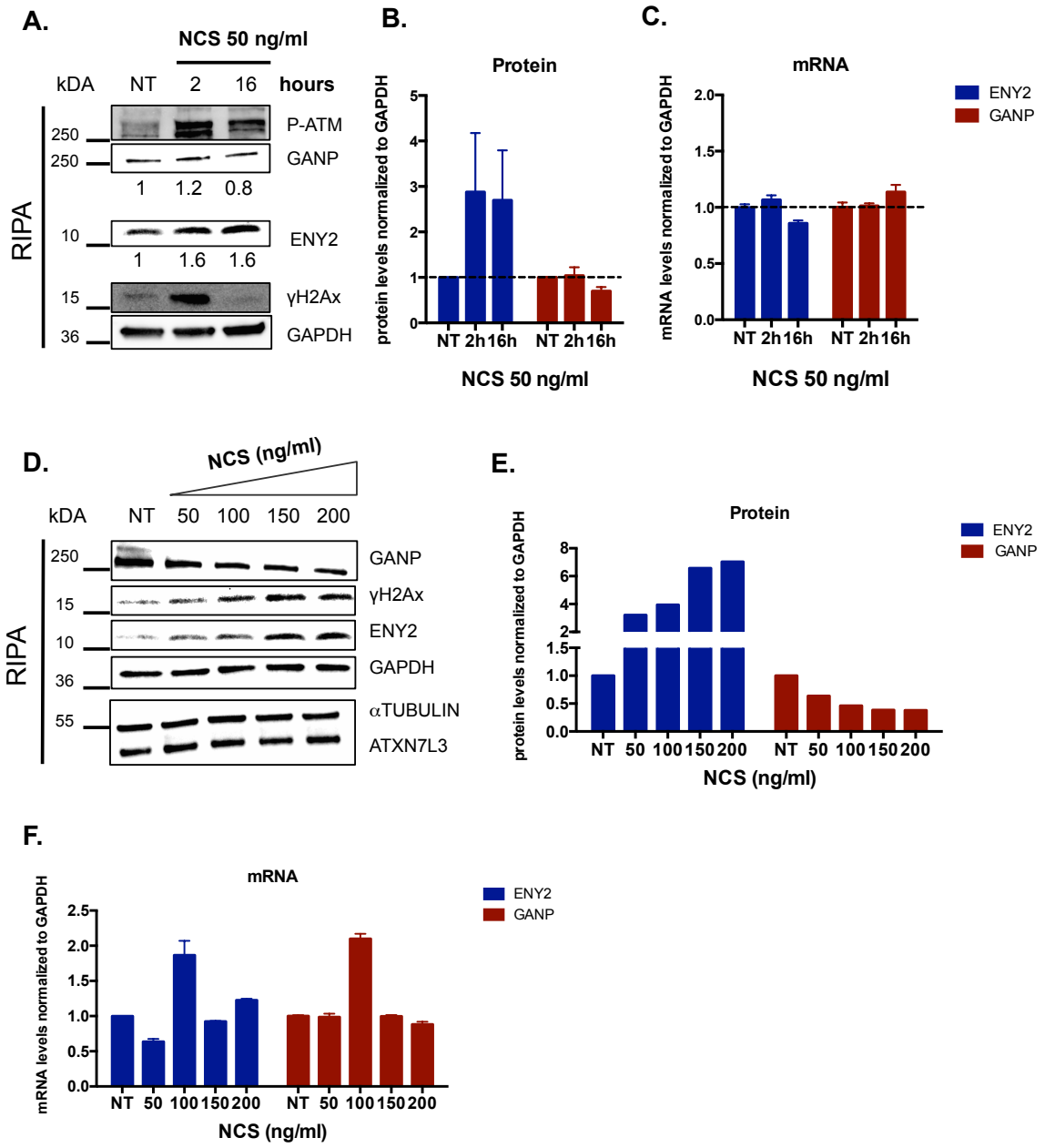


Figure 19 ENY2 is stabilized upon DNA damage

(A) Western blot analysis of total protein extract of asynchronous HeLa cells upon damage induction with NCS. Numbers on top correspond to the hours of repair after NCS treatment and NT corresponds to the untreated sample. Numbers below the blots correspond to the protein levels normalized to GAPDH. (B) Graphs correspond to ENY2 (in blue) and GANP (in red) protein levels normalized to GAPDH as a merge of two independent experiments with standard deviations. (C) RNA levels of ENY2 (in blue) and GANP (in red) corresponding to one of the two experiments in A. Cells were divided in two samples, one subjected to protein extraction for western blot analysis and the other to RNA extraction. Values correspond to RNA levels normalized to GAPDH. Standard deviations were calculated on the basis of two technical replicates. (D) Western blot analysis of total protein extract of asynchronous HeLa cells upon damage induction with NCS. Numbers correspond to the NCS concentrations used and NT corresponds to the untreated. Proteins were extracted after 2 hours of repair after treatment. (E-F) Graphs correspond to ENY2 (in blue) and GANP (in red) protein levels (E) and RNA levels (F) normalized to GAPDH of the experiment shown in (D).

All data have been additionally normalized to the untreated samples (NT) for better visualization of the results.

3.1.2 ENY2 is distributed between cytoplasm and nucleus in asynchronous HeLa cells

In mammalian cells ENY2 is part of at least five different complexes. The association with ATXN7L3B was proposed to take place in the cytoplasm (Li et al., 2016). In the nucleus at least four other populations of ENY2 can be found; in association with TREX-2, stably associating with the NPC (Umlauf et al., 2013) in the SAGA DUB module, and as part of other two DUBs (Atanassov et al., 2016).

To get further insights into ENY2 distribution we conducted salt-based cellular fractionation in asynchronous HeLa cells, to separate the different cellular compartments, followed by western blot analysis (Figure 20A). Following this approach we could detect as expected, some ENY2 in the cytoplasm. Efficient separation of cellular compartments was confirmed by GAPDH, which was almost exclusively present in the cytoplasm fraction. However the majority of ENY2 protein could be extracted with 450 mM salt, that corresponds to chromatin-associated proteins. At 650 mM salt the remaining population of ENY2 could be extracted together with GANP, corresponding to the proteins that strongly associate with chromatin. The efficient chromatin fractionation was verified by Pol II and histone H3 distributions (Figure 20A). However salt based-fractionation does not allow a differentiation between chromatin-associated and NPC-associated proteins, thus the GANP population at 650 mM salt could also correspond to the NPC-associated fraction of GANP.

As shown before ENY2 protein is stabilized after damage induction with NCS. A possible explanation is that it is stabilized on chromatin for H2Bub1 deubiquitination or in the cytoplasm and/or at the NPC to reduce deubiquitination. Thus following the same salt-based fractionation method we investigated a possible shift in ENY2 population upon damage induction with NCS in asynchronous HeLa cells. No significant change was detected following NCS treatment as the majority of ENY2 could be still extracted at 450 mM salt (Figure 20B).

These results indicate that ENY2 populations can be separated in a cytoplasmic pool and a nuclear pool. The latter appears to be constituted by a dynamic chromatin-interacting pool extracted at low salt concentrations (250 mM salt), and a more stable pool, interacting with chromatin and with the NPC, extracted at high salt concentrations. However, although ENY2 protein is stabilized after damage induction, a shift in the populations was difficult to visualize using salt-based fractionation.

Figure 20

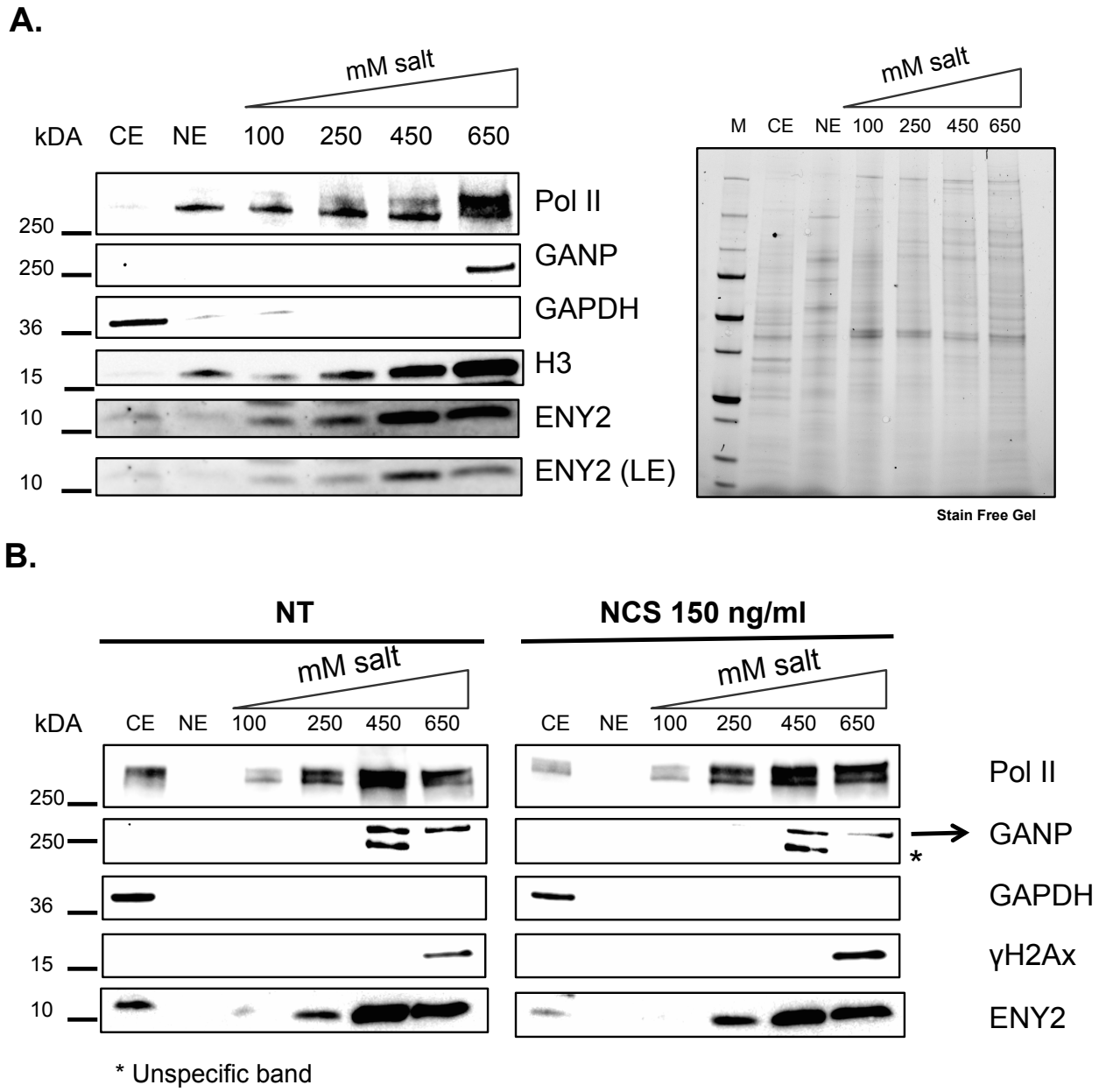


Figure 20 ENY2 is distributed among cytoplasm and nucleus in asynchronous HeLa cells

(A) Western blot analysis of salt-based cellular fractionation of asynchronous HeLa cells. Proteins were extracted using increasing salt concentrations from 100 mM to 650 mM. For normalization equal amount of total proteins were loaded in each well for each fraction. (B) Stain Free gel shows similar amount of total protein loaded. (B) Western blot analysis of salt-based cellular fractionation of asynchronous HeLa cells untreated (NT) or treated with NCS. Extraction of protein was performed after 2 hours of repair after NCS treatment.

LE = Lower exposure; M = marker; CE = cytoplasmic extract; NE = nuclear extract without salt; NT = Untreated

3.1.3 ENY2 is restricted from chromatin upon DNA damage induction in G2 synchronized HeLa cells

Our previous results demonstrated that the balance between H2B and H2Bub1 is important for DNA damage repair by homologous recombination and that depletion of ENY2 and ATXN7L3 as part of the DUB module increases H2Bub1 levels and homologous recombination efficiency. Homologous recombination process is restricted to S and G2 phases of the cell cycle. We showed that ENY2 protein is stabilized after damage in asynchronous cells, however we did not see any shift in the distribution of ENY2 using salt-based fractionation before and after damage induction in these cells. We also showed that ENY2 in the nucleus is progressively extracted from chromatin increasing the salt concentration thus a consistent pool of ENY2 that associates with chromatin exists in human cells, which might represent the DUBs-associated pool. We reasoned that since HR would repair damage induced in the G2 phase of the cell cycle, DUB association with damaged chromatin in G2 phase might be reduced to avoid excessive H2Bub1 deubiquitination, consequently favouring HR.

Thus we analysed the chromatin-associated pool of ENY2 in cells synchronized in late G2 phase of the cell cycle with RO-3066 (Vassilev et al., 2006). Extraction of pure chromatin-associated proteins was achieved by Micrococcal Nuclease (MNase) treatment on the remaining DNA pellet after RIPA extraction of soluble proteins and proteins weakly associated with chromatin. For this purpose we utilize a HeLa cell line overexpressing Human influenza hemagglutinin (HA)-tagged and Green Fluorescent Protein (GFP)-tagged version of ENY2 (Umlauf et al., 2013) to facilitate ENY2 revelation in the chromatin-associated fraction, that might be minimal compared to the nucleoplasmic and cytoplasmic fraction. Proteins with strong chromatin association were successfully separated from the rest as shown by the presence of GAPDH only in the RIPA extraction and H3 only in the MNase digested pellet (Figure 21A). Moreover the RPB1 subunit of Pol II showed a smear only in the MNase pellet that is consistent with the phosphorylated C-terminal domain (CTD) of the active enzyme on chromatin (Figure 21A). In addition GANP was only present in the RIPA extraction suggesting that indeed with this method we can successfully separate chromatin-bound proteins with a minimal contamination of NPC-associated fraction (Figure 21A). Efficient chromatin shearing was analysed by DNA electrophoresis showing DNA fragments of around 150-200 base pairs (data not shown).

Surprisingly ENY2 levels on chromatin bound fraction were decreased upon damage induction with NCS in G2 synchronized cells (Figure 21B), that was consistent upon three

independent experiments (Figure 21C). This is in accordance with the idea that, upon induction of damage in G2, H2Bub1 is required for successful homologous recombination and ENY2, together with the DUB, might be excluded from chromatin to avoid excessive deubiquitination. In asynchronous cells depletion of ENY2 results in a similar effect, stimulating unscheduled HR. However additional experiments are needed to get more insights into ENY2 distribution and regulation in correlation with the role of the different DUBs in DNA repair.

Figure 21

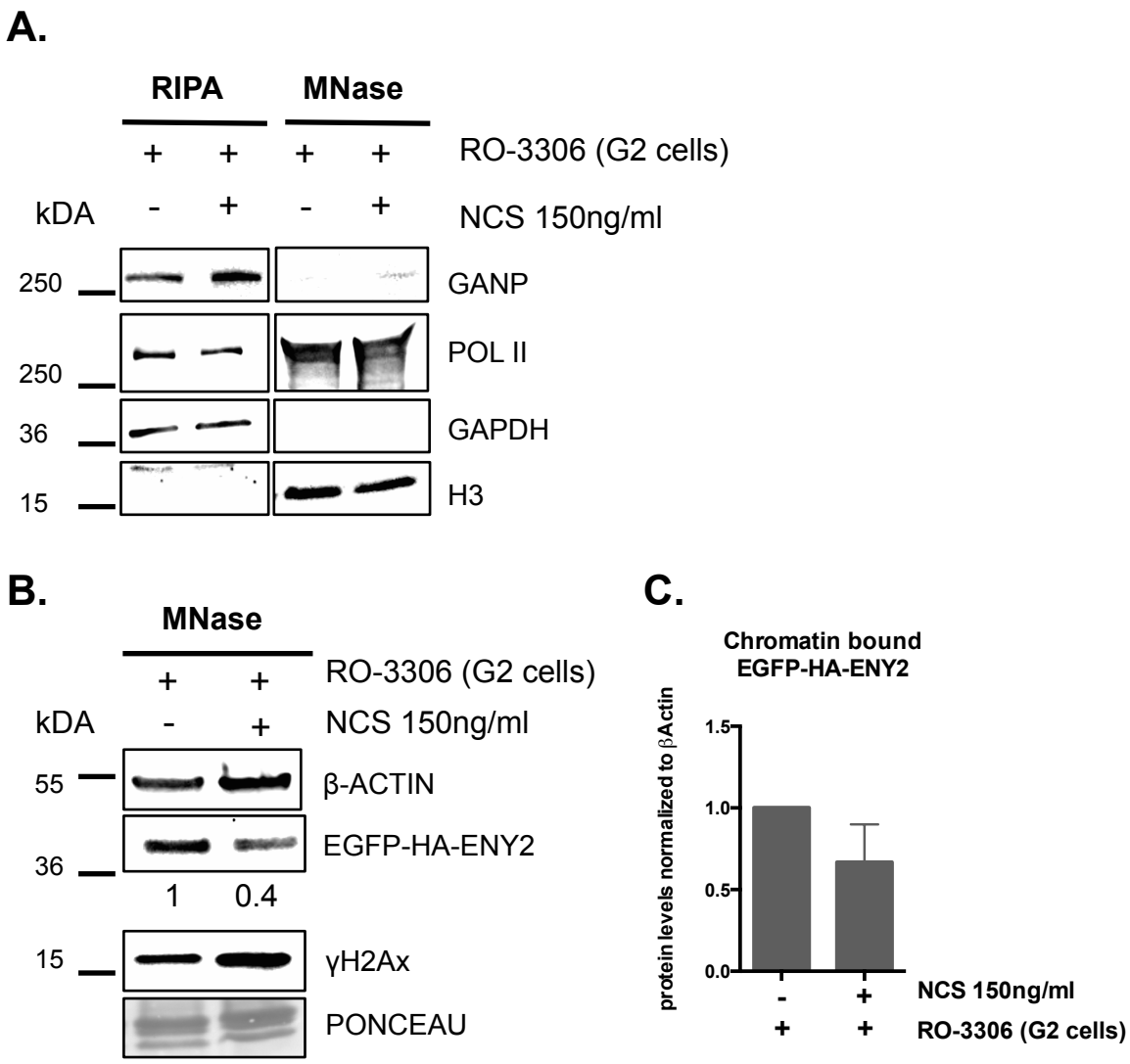


Figure 21 ENY2 is restricted from chromatin upon damage induction in G2 synchronized cells

(A) Western blot analysis of total protein lysate and MNase treated chromatin of G2 synchronized cells using RO-3306, in the absence or presence of damage. Cells were treated with RO-3066, and after 24 hours NCS was added in the medium. Total protein lysate was obtained by RIPA extraction. MNase was added on the chromatin pellet. Efficient separation of total protein and chromatin-associated proteins was verified by GAPDH, Pol II and H3 blot. (B) Western blot of MNase treated chromatin with or without induction of damage with NCS. Numbers correspond to EGFP-HA-ENY2 protein level normalized to β-actin corresponding to the experiment shown in (B). (C) Graph represents the normalization of EGFP-HA-ENY2 protein levels on chromatin as a merge of three independent experiments with standard deviation.

3.1.4 GANP is not redistributed from the NPC to chromatin upon damage

WB analyses did not show any significant change in the levels of GANP upon damage induction (Figure 19A, B) or differences in the distribution upon salt-based fractionation (Figure 20B). Previous experiments have shown no relocation of GANP from the NPC to nuclear interior upon transcription inhibition with α-amanitin or actinomycin D (Umlauf et al., 2013). Note, however, that potentially contrasting results have been obtained in this contest, showing redistribution of GANP upon transcription elongation inhibition with DRB (Wickramasinghe et al., 2010). Thus it seems that GANP distribution in the nuclear environment is not well understood

We sought to determine if any significant relocation of GANP from the NPC to chromatin would be visible, upon massive damage induction with NCS, by performing IF experiments. We treated the cells with NCS at high doses and we let them repair for 2 hours before fixation, conditions in which we detected increased levels of ENY2 by WB analysis. However, also in this case, we were not able to see any difference between control and treated cells in GANP localization at the NPC or intensity. As expected we detected GANP protein as a ring at the nuclear periphery (Figure 22A) that did not change upon NCS treatment (Figure 22A, B). Efficient induction of breaks with NCS was monitored by γH2AX staining.

It is worth mentioning that cells were rapidly treated with pre-extraction buffer prior to fixation to reduce background. This treatment, although was performed for a few seconds, could eliminate nucleoplasmic proteins that might not strongly associate with chromatin or with the NPC, thus if a little nucleoplasmic population of GANP exists it could be lost during pre-extraction. Nevertheless from this experiment we concluded that the population of GANP located at the NPC is highly stable, as previously suggested (Umlauf et al., 2013), and is not relocated from the NPC to damaged chromatin after massive induction of breaks with NCS.

Figure 22

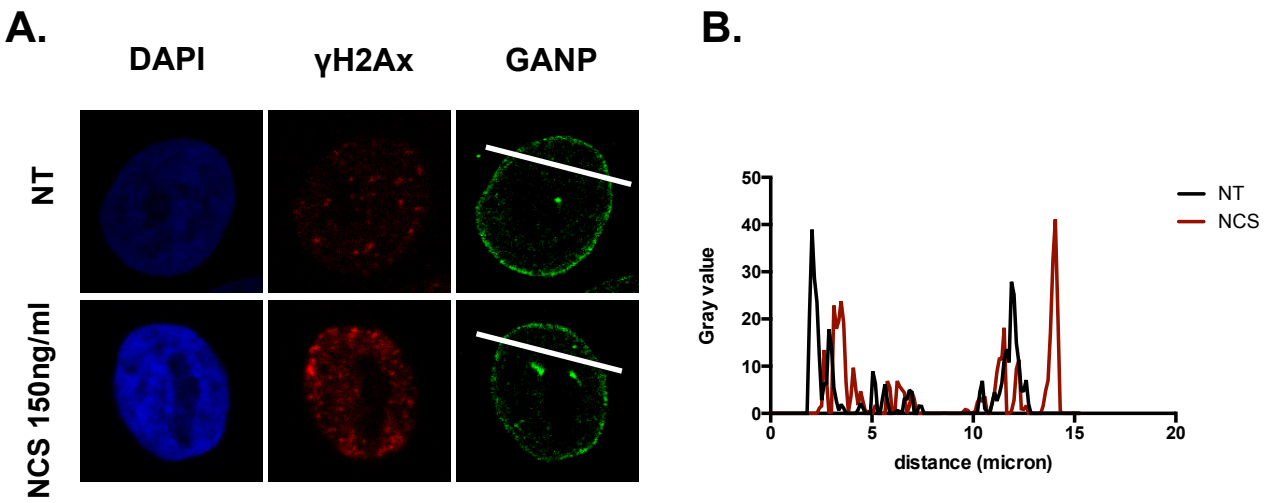


Figure 22 GANP is not recruited on damaged chromatin

(A) Immunofluorescence analysis of representative cells stained with DAPI (in blue), γH2AX (in red) and GANP (in green) in untreated (NT) or NCS-treated cells. Cells were fixed at 2 hours of repair after damage induction with NCS. Images were taken by confocal microscopy. (B) Graph represents the fluorescence distribution for GANP staining along the white line indicated in (A). Black graph represents the GANP distribution in the untreated (NT) sample, and the red line indicates GANP distribution in the NCS treated sample.

3.2 GANP influences the global epigenetic landscape

The scaffold protein of the human TREX-2 complex, GANP, possesses homology with Sac3 protein in yeast. However, as mentioned in the introduction GANP possesses additional functional domains that might account for different roles in human cells. One of these domains is the HAT domain at its C-terminal (Figure 16) that was suggested to acetylate H3 at residues K9 and K27 at the Ig diversification locus in chicken B cells *in vivo* (Singh et al., 2013). Moreover it was shown that cells depleted for one GANP allele (GANP +/- cells) failed to acetylate H4 at the same Ig locus (Eid et al., 2014). However in B cells GANP is overexpressed and it was shown to localize at this locus by ChIP analysis. In other types of somatic cells GANP localizes at the NPC, and previous results showed only a minimal nucleoplasmic pool that could interact with chromatin ((Umlauf et al., 2013) and figure 21A). Interestingly in plants, it was shown that the homologue of ySac3, SAC3B, controls heterochromatin spreading through H3K9me2 levels. However the exact mechanisms remain unrevealed (Yang et al., 2017). Given the presence of a HAT domain in GANP we decided to analyse global histone acetylation in HeLa cells depleted of GANP. After 48 hours of siRNA treatment we performed acidic extraction to enrich for histone proteins. We analysed total H4 acetylation using an antibody detecting acetylated H4 at multiple lysines. We detected a slight decrease in H4 acetylation upon GANP KD compared to control cells in two independent experiments (Figure 23A, B). Moreover we detected a slight decrease in H3 acetylation, using an antibody recognizing H3 acetylated lysines, that was specific for K9, K14, K18, K23 and K27 (Figure 23A, B). To understand if GANP affects acetylation of specific residues we analysed the levels of acetylation at different lysines separately. Surprisingly we detected a reduction in global acetylation of three histone residues, H3K9ac, H3K14ac and H4K16ac upon GANP KD compared to control cells (Figure 23A) while we did not detect changes in H3K18ac (Figure 23A). These results suggest that GANP might be involved in the global acetylation landscape controlling acetylation of different histones in a direct or indirect manner. Histone acetylation is generally considered a mark of open chromatin structure, transcriptionally active, namely euchromatin. Heterochromatin instead is generally characterized by histone methylation. As mentioned before, in plant, SAC3B seems to be involved in controlling heterochromatin spreading through H3K9me2.

H3K9me2 is generated by SUV39 methyltransferase family (Shi and Whetstine, 2007) and demarcates large heterochromatin domains especially gene-deprived regions. Interestingly, as mentioned in the introduction, H3K9me2 domains also strongly correlate

with LADs at the nuclear periphery, where there is enrichment of heterochromatin and inactive genes. To understand if the observed decrease in histone acetylation was accompanied by increase in histone methylation, and considering GANP localization mainly at the nuclear periphery, we decided to specifically analyse H3K9me2 levels performing IF experiments to visualize any possible change of H3K9me2 at the nuclear periphery. However we detected a visible global increase in H3K9me2 in the whole cell nucleus (Figure 23B). Repeated measures of the intensity of H3K9me2 showed that the increase was consistent and significant upon three independent experiments (Figure 23C)
Taken together these results suggest that GANP might be involved in controlling the global epigenetic landscape in human cells. KD of GANP induces reduction of acetylation of different histone marks and a concomitant increase in the heterochromatic mark H3K9me2. This suggests a spreading of heterochromatin over euchromatin in the whole cell nucleus. Further investigations will be required to assess if this is a direct result of the decreased GANP HAT activity, or an indirect effect on specific HATs responsible for histones acetylation. We did not detect substantial levels of GANP in the MNase fraction in G2 cells (Figure 21B), suggesting that it could be an indirect effect, similar to the SAGA/TREX-2 interplay in response to damage.

Figure 23

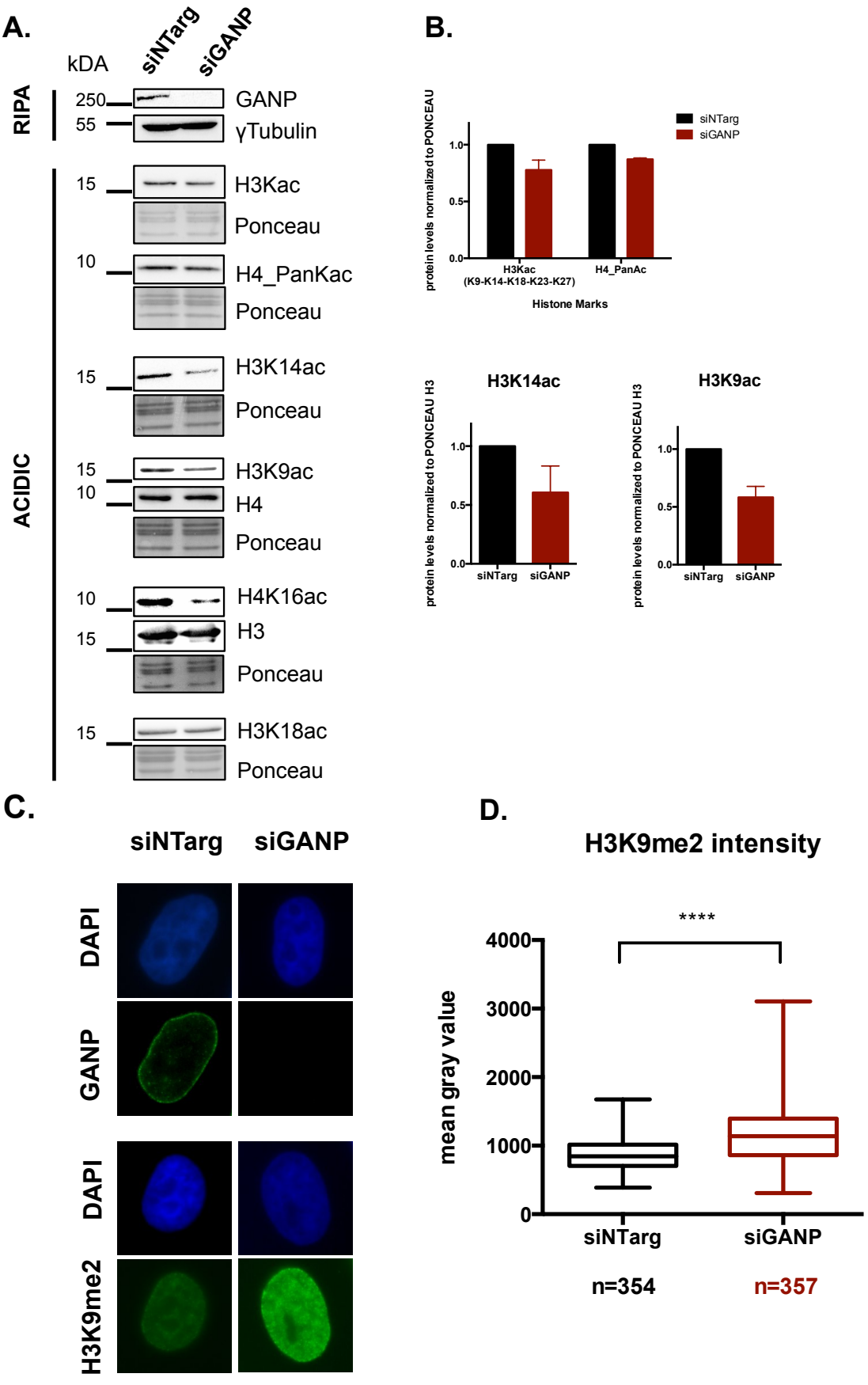

A.
siNTarg
siGANP
kDA
RIPA
250
55
GANP
γTubulin
ACIDIC
15
H3Kac
Ponceau
10
H4_PanKac
Ponceau
15
H3K14ac
Ponceau
15
10
H3K9ac
H4
Ponceau
10
15
H4K16ac
H3
Ponceau
15
H3K18ac
Ponceau
B.
siNTarg
siGANP
protein levels normalized to PONCEAU
1.0
0.5
0.0
H3Kac
(K9-K14-K18-K23-K27)
H4_PanAc
Histone Marks
H3K14ac
protein levels normalized to PONCEAU H3
siNTarg
siGANP
H3K9ac
protein levels normalized to PONCEAU H3
siNTarg
siGANP
C.
siNTarg
siGANP
DAPI
GANP
DAPI
H3K9me2
D.
H3K9me2 intensity
****
mean gray value
4000
3000
2000
1000
0
siNTarg
siGANP
n=354
n=357

Figure 23 GANP regulates global epigenetic landscape

(A) Western blot analysis of RIPA and acidic extraction from HeLa cells depleted of GANP. Acetylation of H3 and H4 different residues was analysed using specific antibodies. (B) Graphs represent the quantification for H3Kac and H4Kac different residues as a merge of two independent experiments with standard deviations. (C) Immunofluorescence analysis of representative cells stained with DAPI (in blue), GANP (in green, upper panel) and H3K9me2 (in green, lower panel). GANP disappearance at the periphery showed successful depletion by siRNA treatment. (D) Box and whiskers min to max graph showing the quantification of the signal intensity for H3K9me2 of three independent experiments. Numbers on the X-axis indicate the number of cells analysed per each condition. Statistical significance was assessed performing t-test. **** $p < 0.0001$

4. DISCUSSION

4.1 TREX-2 and SAGA cooperate to maintain H2B/H2Bub1 balance important for DNA repair by homologous recombination

My experiments have revealed a new role for TREX-2 and SAGA DUB in global DSB repair in human cells. I have shown that depletion of TREX-2 scaffold subunit GANP impacts DSB repair through homologous recombination in a global manner. Previous work has shown that the TREX-2 complex associates with the NPC in a stable way (Umlauf et al., 2013). The NPC is characterized by an open chromatin status that has been shown to be a favourable environment for HR from yeast to humans (Horigome et al., 2014; Lemaitre et al., 2014; Ryu et al., 2015). Moreover, GANP possesses a HAT domain that has been proposed to contribute to the open chromatin status at the IgV locus, important for CSR in human and chicken B cells (Singh et al., 2013; Takei et al., 2001).

Considering all the above-mentioned data, it is reasonable to think that GANP could facilitate homologous recombination repair at the NPC, mainly controlling the repair outcome of peripheral breaks. However our data showed a global defect in DSB repair upon GANP depletion, thus opening two mechanistic possibilities. In one model GANP could be directly recruited at the break site, thus acting in a direct way, while in a second model GANP could affect global genome repair in an indirect way, acting in a signalling cascade that could affect repair.

Basket nucleoporins have been already implicated in DNA repair without being localized at the breaks, acting in a rather indirect way through nuclear import and SUMOylation of 53BP1 (Duheron et al., 2017; Lemaitre et al., 2012; Mackay et al., 2017). Although GANP/TREX-2 association with the NPC depends on these basket nucleoporins, in our work we showed that GANP/TREX-2 acts through a different mechanism. Indeed depletion of GANP/TREX-2 seems not to alter 53BP1 nuclear import or foci formation, but it acts in an indirect way cooperating with the DUB module of the SAGA complex. This cooperation is likely mediated by ENY2, a key subunit of both TREX-2 and the SAGA DUB.

Yeast studies have shown that other subunits of the SAGA complex co-immunoprecipitate with TREX-2 suggesting a physical association of the two complexes (Kohler et al., 2008; Rodriguez-Navarro et al., 2004). Moreover in yeast the role of the TREX-2 complex in maintaining genome stability has been extensively studied. The elucidated mechanism involves defects in mRNA exports with subsequent formation of RNA/DNA hybrids with displacement and accumulation of ssDNA (R-loops), prone to breakage. This mechanism is based on the fact that in yeast transcription and export happen concomitantly, thus blocking export through TREX-2 mutations results in accumulation of RNA in close

proximity to the DNA double helix. Accordingly yeast mutants of almost all TREX-2 subunits, but not SAGA mutants, show hyper-recombination phenotype (Gonzalez-Aguilera et al., 2008)

In my thesis I show that in mammalian cells TREX-2 is fundamental to control SAGA DUB activity although MS data show that other than having a common subunit (ENY2) they do not physically interact (Umlauf et al., 2013). We propose a different mechanism that does not involve export defects, but rather involves chromatin PTMs that play a role in homologous recombination. We show that GANP depletion significantly decreases H2Bub1 global levels. Being the SAGA DUB the major responsible for the removal of H2Bub1, this reduction is likely due to a hyperactivity of the DUB. We showed that indeed the effect could be rescued by inhibiting the DUB in addition to GANP depletion.

As mentioned in the Introduction, the enzyme responsible for ubiquitin removal in the SAGA DUB is USP22, however it has been recently proposed that in USP22 absence other enzymes could take over and form together with ATXN7L3 and ENY2 alternative DUBs that do not interact with SAGA but they could remove ubiquitin from H2Bub1 ((Atanassov et al., 2016) and reviewed in (Helmlinger and Tora, 2017)). For this reason to inhibit the DUB we depleted ENY2 or alternatively ATXN7L3, ensuring to disrupt the functionality of the deubiquitinase, which requires these subunits to be in active conformation.

We propose that depletion of GANP/TREX-2 induces a hyperactivity of the DUB through redistribution of ENY2 from TREX-2 to the DUB. However biochemical analyses have been proven to be challenging considering the unstable nature of ENY2, which is rapidly partially degraded in the absence of GANP. However we cannot exclude a redistribution of newly synthesized ENY2 to the DUB module in the absence of a functional TREX-2. A similar mechanism has been proposed by S. Dent and colleagues regarding the cytoplasmic partner of ENY2, ATXN7L3B, that does not interact with SAGA nor with TREX-2. Although ATXN7L3B depletion has been shown to induce partial ENY2 degradation, its overexpression increases H2Bub1 levels suggesting that ENY2 could be redistributed from the nuclear DUB to the cytoplasm, thus affecting DUB activity and H2Bub1 levels (Li et al., 2016).

In our work we demonstrated an interplay between the DUB and TREX-2 that is important to maintain H2Bub1 levels and affects DNA repair by homologous recombination.

GANP depletion decreases resection, thus RAD51 loading on both genome-wide breaks and at the enzyme-induced single DSB is severely affected. We show that RAD51 loading

in GANP-depleted cells is totally rescued through additional DUB depletion, thus showing that GANP/TREX-2 controls homologous recombination through a process that involves the SAGA DUB and H2Bub1. It has been shown that monoubiquitination of H2B in response to DNA damage is important for recruitment of HR factors (Nakamura et al., 2011), however little is known about the regulation of its deubiquitination. With our work we add a new piece in the regulation of H2B/H2Bub1 balance that involves interplay between two complexes located in different cellular compartments and not physically interacting. We propose that GANP/TREX-2, through ENY2 retention could maintain higher level of H2Bub1 that is needed for homologous recombination. In the absence of TREX-2 the redistribution of ENY2 to the DUB module could contribute to a higher DUB activity/stability and excessive H2Bub1 deubiquitination, that in case of damage would severely decrease homologous recombination efficiency. On the other hand, in the absence of ENY2 and in the absence of ATXN7L3 the DUB stability is severely impaired, inducing a retention of H2Bub1, that in case of damage would increase and stimulate unscheduled homologous recombination (See model in the manuscript).

4.1.1 Regulation of ENY2 distribution is important to ensure efficient repair

Unscheduled homologous recombination might be deleterious for the cells, if happening in the wrong cell cycle stage. We showed an interplay between TREX-2 and the DUB that is likely dependent on the dynamics of ENY2, that can easily stimulate or impair SAGA DUB activity. For this reason ENY2 levels and distribution in the cell should be tightly controlled in the presence of damage. ENY2 is a highly unstable protein, and this probably facilitates its fast regulation. Our additional data showed that ENY2 is stabilized in the presence of damage (Figure 19), however we were unable with salt-based cellular fractionation to show stabilization in a specific cellular compartment (Figure 20). At the same time with this method we were likely unable to differentiate between NPC-associated and chromatin-associated proteins. These experiments were conducted in asynchronous HeLa cells thus in a situation of balance among different repair pathways.

Three possible scenarios can be envisaged; (i) ENY2 could be stabilized on chromatin together with the DUBs, (ii) at the NPC with TREX-2 or (iii) in the cytoplasm in association with ATXN7L3B. We did not detect a change in the cytoplasmic fraction upon salt-based fractionation thus we hypothesized that the stabilization might happen on chromatin or at the NPC having two different outcomes. Stabilization of ENY2 at the NPC would shift ENY2 population from the DUB to TREX-2, thus rising H2Bub1 levels and stimulating HR. ENY2 stabilization on chromatin might happen with two other possible mechanisms; a

stabilization at the sites of breaks, to counteract excessive monoubiquitination, or a stabilization elsewhere to allow again rising of H2Bub1 at the breaks to stimulate HR.
HR is cell cycle dependent thus it is reasonable to speculate that ENY2 distribution might depend on the cell cycle stage, and it might be cell cycle regulated in the presence of damage. This possible mechanism is supported by our results in G2 synchronized cells, where induction of breaks stimulates HR. ENY2 seems to be restricted from chromatin in G2 cells after damage induction and this would be in accordance with the preferential use of HR in G2 phase and the need of H2Bub1 for the recruitment of HR factors (Nakamura et al., 2011).
However further experiments are needed to understand the exact regulation of ENY2 dynamics in response to damage (see perspectives).

4.2 GANP controls global repair and global chromatin epigenetic status

In my thesis manuscript I show that GANP/TREX-2 regulates repair in an indirect way through H2Bub1 levels. GANP is mainly localized at the NPC, however its depletion affects global DSBs repair. Salt-based fractionation showed no redistribution of GANP in different compartments upon global DNA damage induction (Figure 20B) and IF analysis showed no change in the NPC-associated fraction of GANP upon damage induction (Figure 22A). Moreover we did not detect significant GANP amount in MNase treated chromatin extracts (Figure 21A) suggesting that GANP is mainly localized at the NPC.
At the same time our data show that surprisingly GANP seems to have a role in controlling global epigenetic landscape, with a shift from euchromatin-associated marks towards heterochromatin-associated marks, in a damage-independent manner (Figure 23). This is in accordance with the fact that GANP possesses a HAT domain, thus might be involved in histone acetylation, which is a mark of open chromatin structure. Moreover we detected an increase in H3K9me2 that is a mark of silenced chromatin, especially in the LADs present at the nuclear periphery. Surprisingly our immunofluorescence showed a global change in H3K9me2 that was not only specific for the nuclear periphery.
To explain this finding different scenarios are possible. In the first scenario we can speculate that a certain amount of GANP protein might be located in the nucleoplasm and that it might have functions in regulating chromatin structure. It has already been proposed that in B cells GANP is recruited on chromatin at the IgV locus to promote efficient CSR; however in B cells GANP is overexpressed compared to other somatic cell lines and this might facilitate GANP detection. In our case we might not be able to detect a significant

amount of GANP in the chromatin-associated fraction (Figure 21A) but we cannot rule out that a minor population of GANP associates with chromatin.

In the second scenario GANP might affect epigenetic landscape in an indirect way, with a similar mechanism through which it affects repair. GANP could affect the function of specific HATs that are involved in histone acetylation being involved in the regulation of their activity.

However additional experiments will be required to assess how GANP localization is regulated and how it affects such a diversity of histones PTMs (see perspectives).

5. PERSPECTIVES

5.1 TREX-2 and SAGA cooperate to maintain H2B/H2Bub1 balance important for DNA repair by homologous recombination

I have shown in my thesis manuscript that H2B/H2Bub1 balance is important to control repair by homologous recombination and that the interplay between TREX-2 and the SAGA DUB contributes to maintain this balance. Although we demonstrated that GANP/TREX-2 regulation of resection and HR depends on the SAGA DUB, additional experiments might be needed to clarify the exact molecular mechanism of this interplay.

We show global reduction of H2Bub1 in GANP-depleted cells, however ChIP analyses of DUB subunits and/or H2Bub1 at the site of breaks would be needed to elucidate the specificity of the mechanism at the damaged sites. We could speculate that in GANP-depleted cells a higher interaction of the DUB with chromatin would stimulate excessive deubiquitination at the site of breaks, corresponding to lower amount of H2Bub1 and decreased homologous recombination.

Importantly, as mentioned in the introduction, ENY2 was found to be part of two other DUBs that do not associate with SAGA, containing the alternative deubiquitinating enzymes USP27X or USP51. USP51 has been already suggested to deubiquitinate H2AK13/15ub in response to damage (Wang et al., 2016). The activity of these additional DUBs is strictly dependent on the presence of ENY2 and ATXN7L3, thus siRNAs depletion of these adaptors is likely to destabilize not only SAGA DUB but also all the ENY2-containing DUBs. However further experiments will be needed to understand if the activity of these DUBs might be affected by GANP/TREX-2 depletion and could contribute to H2Bub1 regulation to direct homology directed repair.

Our study and previous studies showed that H2Bub1 is important for DNA repair, however the mechanisms through which H2Bub1 operates at DSBs sites are not elucidated yet. It was proposed that the addition of ubiquitin on H2B might induce chromatin relaxation that would be needed to allow recruitment of repair factors (Nakamura et al., 2011). However chromatin relaxation is an early event that happens immediately after damage, while in our data we show that global H2B is still monubiquitinated at 2 hours of repair after NCS treatment in higher levels compared to control cells. In addition the immediate chromatin relaxation is also important for ATM activation (Bakkenist and Kastan, 2003; Rogakou et al., 1999; Rogakou et al., 1998; Stucki and Jackson, 2006) and in the absence of GANP although we detect defective H2Bub1 we do not detect a defective DDR activation and ATM phosphorylation. This suggests that probably there are additional mechanisms through which H2Bub1 might contribute to repair. It cannot be excluded that some repair

factors might interact directly with H2Bub1 at the sites of breaks and until now, although the defective recruitment of factors at the site of breaks has been observed, a direct interaction has not been investigated. Possible elucidation of a putative interaction could come from biochemical *in vitro* approaches using a non-deubiquitinable version of recombinant H2Bub1.

5.1.1 Regulation of ENY2 levels is important to ensure efficient repair

Our additional results suggest that ENY2 is stabilized upon damage induction at the protein level. At the same time we did not detect an increase in ATXN7L3 protein levels. Several possible explanations can be derived by these observations. One explanation could be that ENY2 is not stabilized in a DUB module, or in a second explanation stabilization of the DUB does not necessarily imply stabilization of ATXN7L3. However it is known that ATXN7L3 is rapidly degraded in ENY2 absence thus suggesting a reciprocal regulation. An additional explanation could be stabilization in the cytoplasm in association with ATXN7L3B. We did not detect a difference in ENY2 levels in the cytoplasmic fraction after NCS treatment by salt-based fractionation. However analyses of ATXN7L3B protein or RNA levels are required to gain further knowledge about the regulation of the different ENY2 -containing complexes upon damage induction.

Our results in G2 synchronized cells suggest that the distribution of ENY2 is regulated in a cell cycle dependent manner. However further experiments in synchronized cells are necessary to get further insights into the precise mechanisms. On the other hand, drugs that induce S-phase specific damage (i.e camptothecin) could be used in this perspective to analyse ENY2 dynamics using biochemical approaches.

Moreover, microscopy approaches could be particularly useful to assess the *in vivo* dynamics of the proteins upon damage induction in synchronized cells. A previous study has shown that the TREX-2 complex is extremely stably localized at the NPC. FRAP analysis has shown a recovery rate of 8 hours for ENY2 at the NPC, suggesting a very stable interaction and poor dynamicity (Umlauf et al., 2013). However our current results show that ENY2 is distributed among cytoplasm, NPC and inner nucleus, and suggest a higher dynamicity than previously observed, that might be needed for efficient repair. Thus similar FRAP experiments could be performed in different damage conditions, in different cell cycle stages and in the different cellular compartments, to get more insights into the regulation, distribution and stabilization of ENY2 required for DNA repair.

5.2 GANP controls H2Bub1 levels, global repair and global chromatin epigenetic status

Previous data and our experiments suggest that GANP is mainly localized at the NPC however using salt based cellular fractionation we could not differentiate between NPC-associated and chromatin-associated proteins.

With respect to the nuclear pore, GANP is located at the NPC basket, in a manner dependent on NUP153 and TPR (Umlauf et al., 2013). However several nucleoporins and also NUP153 and TPR have been already shown to have a nucleoplasmic fraction (Daigle et al., 2001; Kalverda et al., 2010; Perez-Garrastachu et al., 2017; Rabut et al., 2004). Thus it is also possible that GANP/TREX-2 has also a nucleoplasmic population. To better characterize the nucleoplasmic fraction of GANP, a marker of a nucleoporin with reduced mobility could be utilized to assess NPC proteins distribution in our fractionation and to compare it with GANP localization.

Biochemical approaches using western blot analyses might not be sensitive enough to detect GANP populations in the nucleoplasm. Western blot analyses are based on antibody-specificity which might be hindered by eventual PTMs on GANP. Thus more sensitive techniques such as MS could be used to detect GANP in the different compartments after cellular fractionation.

To understand if GANP regulates the epigenetic landscape in a direct or indirect way complementation experiments could be performed. GANP protein contains different functional domains (Figure 16), a Nups-homology domain at the N-terminal and a C-terminal HAT domain. Different GANP recombinant constructs missing one or the other domain could be used to understand if GANP localization at the NPC, or GANP HAT activity are the driving cause of the epigenetic changes observed in GANP depletion.

6. METHODS

6.1 Cell culture and treatments

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) 1g/l glucose, containing 5% Fetal Calf Serum (FCS) and gentamycin. EGFP-HA-ENY2 cell line was described elsewhere (Umlauf et al., 2013). For treatments, NCS (Neocarzinostatin from *Streptomyces carzinostaticus* N9162 Sigma Aldrich) was added in the medium at a final concentration of 50, 100, 150 or 200 ng/ml (see Figures' legend). NCS treatment was performed for 15 minutes at 37°C. Medium was refreshed to allow cells to repair for different time points (see Figures' legends) before fixation or protein extraction. For G2 synchronization CDK1 inhibitor RO-3660 (MERCK chemicals LTD) was added in the medium at a final concentration of 10 µM for 24 hours. If additional NCS treatment was performed on G2 synchronized cells, NCS was added in the RO-3306-containing medium that was not refreshed after 15 minutes to avoid exit from G2 phase of the cell cycle. siRNAs transfection was performed with Lipofectamine 2000 (Lipofectamine® 2000 Thermo Fisher Scientific) following manufacturer's instructions. siRNAs treatment was performed for 48 hours. For siRNAs list refer to the manuscript "*a regulated interplay between the human TREX-2 mRNA export complex and the SAGA DUB module is required for efficient DNA damage repair*" in results' section 3.1.

6.2 Cellular extracts

Cells were harvested after treatments in PBS 1X. Total protein lysate was obtained by adding cold RIPA buffer (Cold Spring Harbor Protocols) on the cell pellet. Histone proteins extract was obtained by adding acidic buffer (10 mM HEPES pH. 7.9; 1.5 mM $MgCl_2$; 10 mM KCl; 0.5 mM DTT; 0.2 M HCl) on cell pellet. 1X Proteinase Inhibitor Cocktail (PIC, cOmplete™, Mini EDTA free, Proteinase Inhibitor Cocktail 11836170001 Roche), Phosphatase Inhibitor Cocktail (PhosSTOP™ PHOSS-RO Roche) and 10 mM N-Ethylmaleimide (NEM E3876 SIGMA-ALDRICH) were added in all buffers.

Salt-based cellular fractionation was performed using the following procedure. Cells were harvested in PBS 1X containing PIC 1X and cytoplasmic extract (CE) was obtained by adding hypotonic buffer (20 mM HEPES pH 7; 20 mM NaCl; 5 mM $MgCl_2$) on cell pellet and douncing 10 times with Dounce B. Cells were centrifuged and the supernatant was retained as cytoplasmic extract. Nuclear soluble extract (NE) was obtained by resuspending nuclei in hypotonic buffer with 0.5% of NP-40 (IGEPAL® CA-630 I3021 Sigma-Aldrich). Nuclei were centrifuged and the supernatant was retained as NE.

Subsequent extractions of chromatin bound proteins were obtained by increasing the level of NaCl concentration in the hypotonic buffer, leaving in ice for 15 minutes and centrifuging 800 g for 5 minutes at 4°C. 1X PIC and 1X phosphatase inhibitor cocktail was added in all buffers.

Extraction of proteins that strongly associate with chromatin was obtained with the following procedure: total protein lysate was obtained by RIPA extraction (as before) and the remaining viscous pellet, mainly containing chromatin, was resuspended in Sucrose buffer (20 mM TRIS pH 7.6; 15 mM KCl; 60 mM NaCl; 0.34 M Sucrose; 0.15 mM Spermine; 0.5 mM Spermidine) additioned with 1 mM $CaCl_2$. MNase (Micrococcal Nuclease ^{TM} Thermo Scientific, 300 U/µl) was added at the final concentration of 1 U/µl and incubated for 15 minutes at 37°C under agitation. Digestion was stopped adding 4 mM EDTA and incubating in ice for 5 minutes. 1X PIC was added in all buffers. DNA was extracted with phenol-chloroform and run on a 1% agarose gel to confirm efficient chromatin shearing (data not shown).

Samples were run on 4–20% Precast gels (Mini-PROTEAN® TGX Stain-Free™ Protein Gels, Biorad) and membranes blotted with indicated antibodies (for antibodies list see below). Protein levels were quantified using Image J.

6.3 RNA extracts and qPCR

RNA was extracted using NucleoSpin® RNA Kit (Macherey-Nagel) and cDNA was obtained with Superscript™ II Reverse Transcriptase (Invitrogen) following manufacturer's instructions. qPCR was performed using LightCycler® 480 SYBR Green I Master (Roche) following manufacturer's instructions.

6.4 Immunofluorescence

HeLa cells were plated in 24 well plates on round cover glasses (VWR®). At the decided time point or confluence cells were washed carefully with PBS 1X. Cells immunostained for GANP were treated with pre-extraction buffer (0.5% triton X; 50 mM HEPES pH 7; 150 mM NaCl; 10 mM EGTA; 2 mM $MgCl_2$) for 10 seconds before fixation with Paraformaldehyde (PFA) 4% for 10 minutes at RT. Cells immunostained for H3K9me2 were fixed with PFA immediately after washing. After fixation cells were washed with PBS 1X and permeabilized with sterile permeabilization buffer (0.3% Triton X) for 10 minutes at RT, and blocked with sterile 5% BSA in PBS 1X for 1 hours. Primary antibodies (GANP,

2988 in house (Umlauf et al., 2013), and H3K9me2 were added for one hour, cells were washed and stained with secondary fluorescent antibody (Alexa Fluor 488 Goat anti-Rabbit IgG, Life Technology, A-11008). DAPI (Sigma Aldrich D9542) was added at 0.3 mM final concentration for 2 minutes and coverslips were mounted using ProLong® Gold Antifade Mountant (Life Technologies, P36934). Images were acquired using the fluroescence microscope Leica DM 4000 B or Confocal microscope Leica TCS SP5 Inverted. Images were analysed using Image J.

6.5 Antibodies' list

Antibody	Reference	Working dilution
Anti-GANP	2988 in house (Umlauf et al., 2013)	1:500 (Immunofluorescence)
Anti-H4K16ac	Active Motif (39167)	1:1000 (Western blot)
Anti-H3K9me2	Cell signalling (#9753)	1:1000 (Immunofluorescence)
Anti-H3K18ac	Cell signalling (#9675)	1:1000 (Western blot)
Anti-H4Kacetyle	Active Motif (39926)	1:1000 (Western blot)
Anti-H3Kacetyle	Abcam (ab47915)	1:1000 (Western blot)
Anti-H3K9ac	Merck Millipore (07-352)	1:1000 (Western blot)
Anti-H3	Abcam (ab1971)	1:10000 (Western blot)
Anti-H4	Abcam (ab7311)	1:10000 Western blot
Anti-H3K14ac	Millipore (07-353)	1:5000 (Western blot)
Anti-ATXN7L3	2325 in house	1:1000 (Western blot)
Anti-Pol II	7C2 in house	1:5000 (Western blot)
Anti-β−ACTIN	Sigma Aldrich (A5441)	1:1000 (Western blot)
Anti-HA	Sigma Aldrich (3F10)	1:500 (Western Blot)

For additional antibodies, siRNAs and primers used see Materials and Methods section in the manuscript "*A regulated interplay between the human TREX-2 mRNA export complex and the SAGA DUB module is required for efficient DNA damage repair*" in Results section 3.1.

7. REFERENCES

Abe, E., Kuwahara, K., Yoshida, M., Suzuki, M., Terasaki, H., Matsuo, Y., Takahashi, E.I., and Sakaguchi, N. (2000). Structure, expression, and chromosomal localization of the human gene encoding a germinal center-associated nuclear protein (GANP) that associates with MCM3 involved in the initiation of DNA replication. Gene *255*, 219-227.

Ahnesorg, P., Smith, P., and Jackson, S.P. (2006). XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell *124*, 301-313.

Aibara, S., Bai, X.C., and Stewart, M. (2016). The Sac3 TPR-like region in the Saccharomyces cerevisiae TREX-2 complex is more extensive but independent of the CID region. Journal of structural biology *195*, 316-324.

Alt, F.W., Zhang, Y., Meng, F.L., Guo, C., and Schwer, B. (2013). Mechanisms of programmed DNA lesions and genomic instability in the immune system. Cell *152*, 417-429.

An, J., Huang, Y.C., Xu, Q.Z., Zhou, L.J., Shang, Z.F., Huang, B., Wang, Y., Liu, X.D., Wu, D.C., and Zhou, P.K. (2010). DNA-PKcs plays a dominant role in the regulation of H2AX phosphorylation in response to DNA damage and cell cycle progression. BMC molecular biology *11*, 18.

Antony, E., Tomko, E.J., Xiao, Q., Krejci, L., Lohman, T.M., and Ellenberger, T. (2009). Srs2 disassembles Rad51 filaments by a protein-protein interaction triggering ATP turnover and dissociation of Rad51 from DNA. Molecular cell *35*, 105-115.

Atanassov, B.S., Mohan, R.D., Lan, X., Kuang, X., Lu, Y., Lin, K., McIvor, E., Li, W., Zhang, Y., Florens, L.*, et al.* (2016). ATXN7L3 and ENY2 Coordinate Activity of Multiple H2B Deubiquitinases Important for Cellular Proliferation and Tumor Growth. Molecular cell *62*, 558-571.

Audebert, M., Salles, B., and Calsou, P. (2004). Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. The Journal of biological chemistry *279*, 55117-55126.

Aylon, Y., Liefshitz, B., and Kupiec, M. (2004). The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle. The EMBO journal *23*, 4868-4875.

Ayoub, N., Rajendra, E., Su, X., Jeyasekharan, A.D., Mahen, R., and Venkitaraman, A.R. (2009). The carboxyl terminus of Brca2 links the disassembly of Rad51 complexes to mitotic entry. Current biology : CB *19*, 1075-1085.

Bakkenist, C.J., and Kastan, M.B. (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature *421*, 499-506.

Ball, H.L., Ehrhardt, M.R., Mordes, D.A., Glick, G.G., Chazin, W.J., and Cortez, D. (2007). Function of a conserved checkpoint recruitment domain in ATRIP proteins. Molecular and cellular biology *27*, 3367-3377.

Baptista, T., Grunberg, S., Minoungou, N., Koster, M.J.E., Timmers, H.T.M., Hahn, S., Devys, D., and Tora, L. (2017). SAGA Is a General Cofactor for RNA Polymerase II Transcription. Molecular cell.

Bassing, C.H., Chua, K.F., Sekiguchi, J., Suh, H., Whitlow, S.R., Fleming, J.C., Monroe, B.C., Ciccone, D.N., Yan, C., Vlasakova, K.*, et al.* (2002). Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proceedings of the National Academy of Sciences of the United States of America *99*, 8173-8178.

Baudat, F., Imai, Y., and de Massy, B. (2013). Meiotic recombination in mammals: localization and regulation. Nature reviews Genetics *14*, 794-806.

Bauer, A., and Kolling, R. (1996). Characterization of the SAC3 gene of Saccharomyces cerevisiae. Yeast *12*, 965-975.

Beck, C., Boehler, C., Guirouilh Barbat, J., Bonnet, M.E., Illuzzi, G., Ronde, P., Gauthier, L.R., Magroun, N., Rajendran, A., Lopez, B.S.*, et al.* (2014a). PARP3 affects the

relative contribution of homologous recombination and nonhomologous end-joining pathways. Nucleic acids research *42*, 5616-5632.

Beck, C., Robert, I., Reina-San-Martin, B., Schreiber, V., and Dantzer, F. (2014b). Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. Experimental cell research *329*, 18-25.

Bekker-Jensen, S., and Mailand, N. (2010). Assembly and function of DNA double-strand break repair foci in mammalian cells. DNA repair *9*, 1219-1228.

Bertocci, B., De Smet, A., Weill, J.C., and Reynaud, C.A. (2006). Nonoverlapping functions of DNA polymerases mu, lambda, and terminal deoxynucleotidyltransferase during immunoglobulin V(D)J recombination in vivo. Immunity *25*, 31-41.

Bhargava, R., Onyango, D.O., and Stark, J.M. (2016). Regulation of Single-Strand Annealing and its Role in Genome Maintenance. Trends in genetics : TIG *32*, 566-575.

Bhattacharyya, A., Ear, U.S., Koller, B.H., Weichselbaum, R.R., and Bishop, D.K. (2000). The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. The Journal of biological chemistry *275*, 23899-23903.

Blobel, G. (1985). Gene gating: a hypothesis. Proceedings of the National Academy of Sciences of the United States of America *82*, 8527-8529.

Bonnet, J., Wang, C.Y., Baptista, T., Vincent, S.D., Hsiao, W.C., Stierle, M., Kao, C.F., Tora, L., and Devys, D. (2014). The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription. Genes & development *28*, 1999-2012.

Botuyan, M.V., Lee, J., Ward, I.M., Kim, J.E., Thompson, J.R., Chen, J., and Mer, G. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. Cell *127*, 1361-1373.

Boubnov, N.V., Hall, K.T., Wills, Z., Lee, S.E., He, D.M., Benjamin, D.M., Pulaski, C.R., Band, H., Reeves, W., Hendrickson, E.A.*, et al.* (1995). Complementation of the ionizing radiation sensitivity, DNA end binding, and V(D)J recombination defects of double-strand break repair mutants by the p86 Ku autoantigen. Proceedings of the National Academy of Sciences of the United States of America *92*, 890-894.

Boulton, S.J., and Jackson, S.P. (1996). Identification of a Saccharomyces cerevisiae Ku80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. Nucleic acids research *24*, 4639-4648.

Bouwman, P., Aly, A., Escandell, J.M., Pieterse, M., Bartkova, J., van der Gulden, H., Hiddingh, S., Thanasoula, M., Kulkarni, A., Yang, Q.*, et al.* (2010). 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nature structural & molecular biology *17*, 688-695.

Brand, M., Yamamoto, K., Staub, A., and Tora, L. (1999). Identification of TATA-binding protein-free TAFII-containing complex subunits suggests a role in nucleosome acetylation and signal transduction. The Journal of biological chemistry *274*, 18285-18289.

Bryant, H.E., Petermann, E., Schultz, N., Jemth, A.S., Loseva, O., Issaeva, N., Johansson, F., Fernandez, S., McGlynn, P., and Helleday, T. (2009). PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. The EMBO journal *28*, 2601-2615.

Brzovic, P.S., Lissounov, A., Christensen, D.E., Hoyt, D.W., and Klevit, R.E. (2006). A UbcH5/ubiquitin noncovalent complex is required for processive BRCA1-directed ubiquitination. Molecular cell *21*, 873-880.

Buck, D., Malivert, L., de Chasseval, R., Barraud, A., Fondaneche, M.C., Sanal, O., Plebani, A., Stephan, J.L., Hufnagel, M., le Deist, F.*, et al.* (2006). Cernunnos, a novel

nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. Cell *124*, 287-299.
Bugreev, D.V., Yu, X., Egelman, E.H., and Mazin, A.V. (2007). Novel pro- and anti-recombination activities of the Bloom's syndrome helicase. Genes & development *21*, 3085-3094.
Bunting, S.F., Callen, E., Wong, N., Chen, H.T., Polato, F., Gunn, A., Bothmer, A., Feldhahn, N., Fernandez-Capetillo, O., Cao, L.*, et al.* (2010). 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell *141*, 243-254.
Burma, S., Chen, B.P., Murphy, M., Kurimasa, A., and Chen, D.J. (2001). ATM phosphorylates histone H2AX in response to DNA double-strand breaks. The Journal of biological chemistry *276*, 42462-42467.
Butler, L.R., Densham, R.M., Jia, J., Garvin, A.J., Stone, H.R., Shah, V., Weekes, D., Festy, F., Beesley, J., and Morris, J.R. (2012). The proteasomal de-ubiquitinating enzyme POH1 promotes the double-strand DNA break response. The EMBO journal *31*, 3918-3934.
Cabal, G.G., Genovesio, A., Rodriguez-Navarro, S., Zimmer, C., Gadal, O., Lesne, A., Buc, H., Feuerbach-Fournier, F., Olivo-Marin, J.C., Hurt, E.C.*, et al.* (2006). SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. Nature *441*, 770-773.
Cahill, D., and Carney, J.P. (2007). Dimerization of the Rad50 protein is independent of the conserved hook domain. Mutagenesis *22*, 269-274.
Cannavo, E., and Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. Nature *514*, 122-125.
Cantor, S.B., Bell, D.W., Ganesan, S., Kass, E.M., Drapkin, R., Grossman, S., Wahrer, D.C., Sgroi, D.C., Lane, W.S., Haber, D.A.*, et al.* (2001). BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell *105*, 149-160.
Cao, L., Xu, X., Bunting, S.F., Liu, J., Wang, R.H., Cao, L.L., Wu, J.J., Peng, T.N., Chen, J., Nussenzweig, A.*, et al.* (2009). A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. Molecular cell *35*, 534-541.
Carreira, A., Hilario, J., Amitani, I., Baskin, R.J., Shivji, M.K., Venkitaraman, A.R., and Kowalczykowski, S.C. (2009). The BRC repeats of BRCA2 modulate the DNA-binding selectivity of RAD51. Cell *136*, 1032-1043.
Carreira, A., and Kowalczykowski, S.C. (2011). Two classes of BRC repeats in BRCA2 promote RAD51 nucleoprotein filament function by distinct mechanisms. Proceedings of the National Academy of Sciences of the United States of America *108*, 10448-10453.
Casolari, J.M., Brown, C.R., Komili, S., West, J., Hieronymus, H., and Silver, P.A. (2004). Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell *117*, 427-439.
Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Sedelnikova, O.A., Reina-San-Martin, B., Coppola, V., Meffre, E., Difilippantonio, M.J.*, et al.* (2002). Genomic instability in mice lacking histone H2AX. Science *296*, 922-927.
Chan, S.H., Yu, A.M., and McVey, M. (2010). Dual roles for DNA polymerase theta in alternative end-joining repair of double-strand breaks in Drosophila. PLoS genetics *6*, e1001005.
Chang, H.H., and Lieber, M.R. (2016). Structure-Specific nuclease activities of Artemis and the Artemis: DNA-PKcs complex. Nucleic acids research *44*, 4991-4997.

Chang, H.H., Watanabe, G., and Lieber, M.R. (2015). Unifying the DNA end-processing roles of the artemis nuclease: Ku-dependent artemis resection at blunt DNA ends. The Journal of biological chemistry *290*, 24036-24050.

Chang, H.H.Y., Pannunzio, N.R., Adachi, N., and Lieber, M.R. (2017). Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nature reviews Molecular cell biology *18*, 495-506.

Chapman, J.R., Sossick, A.J., Boulton, S.J., and Jackson, S.P. (2012). BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. Journal of cell science *125*, 3529-3534.

Chekanova, J.A., Abruzzi, K.C., Rosbash, M., and Belostotsky, D.A. (2008). Sus1, Sac3, and Thp1 mediate post-transcriptional tethering of active genes to the nuclear rim as well as to non-nascent mRNP. Rna *14*, 66-77.

Chen, J., Silver, D.P., Walpita, D., Cantor, S.B., Gazdar, A.F., Tomlinson, G., Couch, F.J., Weber, B.L., Ashley, T., Livingston, D.M*., et al.* (1998). Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Molecular cell *2*, 317-328.

Chen, L., Nievera, C.J., Lee, A.Y., and Wu, X. (2008). Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. The Journal of biological chemistry *283*, 7713-7720.

Chiolo, I., Minoda, A., Colmenares, S.U., Polyzos, A., Costes, S.V., and Karpen, G.H. (2011). Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell *144*, 732-744.

Choi, J.H., Lindsey-Boltz, L.A., Kemp, M., Mason, A.C., Wold, M.S., and Sancar, A. (2010). Reconstitution of RPA-covered single-stranded DNA-activated ATR-Chk1 signaling. Proceedings of the National Academy of Sciences of the United States of America *107*, 13660-13665.

Christensen, D.E., Brzovic, P.S., and Klevit, R.E. (2007). E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. Nature structural & molecular biology *14*, 941-948.

Coleman, K.A., and Greenberg, R.A. (2011). The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. The Journal of biological chemistry *286*, 13669-13680.

Cooper, E.M., Cutcliffe, C., Kristiansen, T.Z., Pandey, A., Pickart, C.M., and Cohen, R.E. (2009). K63-specific deubiquitination by two JAMM/MPN+ complexes: BRISC-associated Brcc36 and proteasomal Poh1. The EMBO journal *28*, 621-631.

Daigle, N., Beaudouin, J., Hartnell, L., Imreh, G., Hallberg, E., Lippincott-Schwartz, J., and Ellenberg, J. (2001). Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. The Journal of cell biology *154*, 71-84.

Davies, O.R., and Pellegrini, L. (2007). Interaction with the BRCA2 C terminus protects RAD51-DNA filaments from disassembly by BRC repeats. Nature structural & molecular biology *14*, 475-483.

Della-Maria, J., Zhou, Y., Tsai, M.S., Kuhnlein, J., Carney, J.P., Paull, T.T., and Tomkinson, A.E. (2011). Human Mre11/human Rad50/Nbs1 and DNA ligase IIIalpha/XRCC1 protein complexes act together in an alternative nonhomologous end joining pathway. The Journal of biological chemistry *286*, 33845-33853.

Densham, R.M., Garvin, A.J., Stone, H.R., Strachan, J., Baldock, R.A., Daza-Martin, M., Fletcher, A., Blair-Reid, S., Beesley, J., Johal, B*., et al.* (2016). Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection. Nature structural & molecular biology *23*, 647-655.

Derheimer, F.A., and Kastan, M.B. (2010). Multiple roles of ATM in monitoring and maintaining DNA integrity. FEBS letters *584*, 3675-3681.

Deriano, L., Stracker, T.H., Baker, A., Petrini, J.H., and Roth, D.B. (2009). Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. Molecular cell *34*, 13-25.

Doil, C., Mailand, N., Bekker-Jensen, S., Menard, P., Larsen, D.H., Pepperkok, R., Ellenberg, J., Panier, S., Durocher, D., Bartek, J.*, et al.* (2009). RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell *136*, 435-446.

Dong, Y., Hakimi, M.A., Chen, X., Kumaraswamy, E., Cooch, N.S., Godwin, A.K., and Shiekhattar, R. (2003). Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. Molecular cell *12*, 1087-1099.

Duheron, V., Nilles, N., Pecenko, S., Martinelli, V., and Fahrenkrog, B. (2017). Localisation of Nup153 and SENP1 to nuclear pore complexes is required for 53BP1-mediated DNA double-strand break repair. Journal of cell science *130*, 2306-2316.

Eid, M.M., Maeda, K., Almofty, S.A., Singh, S.K., Shimoda, M., and Sakaguchi, N. (2014). GANP regulates the choice of DNA repair pathway by DNA-PKcs interaction in AID-dependent IgV region diversification. Journal of immunology *192*, 5529-5539.

Eliezer, Y., Argaman, L., Rhie, A., Doherty, A.J., and Goldberg, M. (2009). The direct interaction between 53BP1 and MDC1 is required for the recruitment of 53BP1 to sites of damage. The Journal of biological chemistry *284*, 426-435.

Esashi, F., Galkin, V.E., Yu, X., Egelman, E.H., and West, S.C. (2007). Stabilization of RAD51 nucleoprotein filaments by the C-terminal region of BRCA2. Nature structural & molecular biology *14*, 468-474.

Escribano-Diaz, C., Orthwein, A., Fradet-Turcotte, A., Xing, M., Young, J.T., Tkac, J., Cook, M.A., Rosebrock, A.P., Munro, M., Canny, M.D.*, et al.* (2013). A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. Molecular cell *49*, 872-883.

Faza, M.B., Kemmler, S., Jimeno, S., Gonzalez-Aguilera, C., Aguilera, A., Hurt, E., and Panse, V.G. (2009). Sem1 is a functional component of the nuclear pore complex-associated messenger RNA export machinery. The Journal of cell biology *184*, 833-846.

Feller, C., Forne, I., Imhof, A., and Becker, P.B. (2015). Global and specific responses of the histone acetylome to systematic perturbation. Molecular cell *57*, 559-571.

Ferguson, D.O., and Holloman, W.K. (1996). Recombinational repair of gaps in DNA is asymmetric in Ustilago maydis and can be explained by a migrating D-loop model. Proceedings of the National Academy of Sciences of the United States of America *93*, 5419-5424.

Fischer, T., Rodriguez-Navarro, S., Pereira, G., Racz, A., Schiebel, E., and Hurt, E. (2004). Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. Nature cell biology *6*, 840-848.

Fischer, T., Strasser, K., Racz, A., Rodriguez-Navarro, S., Oppizzi, M., Ihrig, P., Lechner, J., and Hurt, E. (2002). The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. The EMBO journal *21*, 5843-5852.

Fradet-Turcotte, A., Canny, M.D., Escribano-Diaz, C., Orthwein, A., Leung, C.C., Huang, H., Landry, M.C., Kitevski-LeBlanc, J., Noordermeer, S.M., Sicheri, F.*, et al.* (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature *499*, 50-54.

Fricke, W.M., and Brill, S.J. (2003). Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes & development *17*, 1768-1778.

Fujimura, S., Xing, Y., Takeya, M., Yamashita, Y., Ohshima, K., Kuwahara, K., and Sakaguchi, N. (2005). Increased expression of germinal center-associated nuclear protein RNA-primase is associated with lymphomagenesis. Cancer research *65*, 5925-5934.

Gagne, J.P., Hendzel, M.J., Droit, A., and Poirier, G.G. (2006). The expanding role of poly(ADP-ribose) metabolism: current challenges and new perspectives. Current opinion in cell biology *18*, 145-151.

Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K.M., and Jackson, S.P. (2009). Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature *462*, 935-939.

Gallardo, M., and Aguilera, A. (2001). A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination. Genetics *157*, 79-89.

Gallardo, M., Luna, R., Erdjument-Bromage, H., Tempst, P., and Aguilera, A. (2003). Nab2p and the Thp1p-Sac3p complex functionally interact at the interface between transcription and mRNA metabolism. The Journal of biological chemistry *278*, 24225-24232.

Garcia, V., Phelps, S.E., Gray, S., and Neale, M.J. (2011). Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. Nature *479*, 241-244.

Garcia-Oliver, E., Garcia-Molinero, V., and Rodriguez-Navarro, S. (2012). mRNA export and gene expression: the SAGA-TREX-2 connection. Biochimica et biophysica acta *1819*, 555-565.

Gasior, S.L., Wong, A.K., Kora, Y., Shinohara, A., and Bishop, D.K. (1998). Rad52 associates with RPA and functions with rad55 and rad57 to assemble meiotic recombination complexes. Genes & development *12*, 2208-2221.

Gatti, M., Pinato, S., Maspero, E., Soffientini, P., Polo, S., and Penengo, L. (2012). A novel ubiquitin mark at the N-terminal tail of histone H2As targeted by RNF168 ubiquitin ligase. Cell cycle *11*, 2538-2544.

Gatz, S.A., Salles, D., Jacobsen, E.M., Dork, T., Rausch, T., Aydin, S., Surowy, H., Volcic, M., Vogel, W., Debatin, K.M.*, et al.* (2016). MCM3AP and POMP Mutations Cause a DNA-Repair and DNA-Damage-Signaling Defect in an Immunodeficient Child. Human mutation *37*, 257-268.

Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M.*, et al.* (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature *415*, 141-147.

Gay, S., and Foiani, M. (2015). Nuclear envelope and chromatin, lock and key of genome integrity. International review of cell and molecular biology *317*, 267-330.

Georgiev, P.G. (1994). Identification of mutations in three genes that interact with zeste in the control of white gene expression in Drosophila melanogaster. Genetics *138*, 733-739.

Georgieva, S., Nabirochkina, E., Dilworth, F.J., Eickhoff, H., Becker, P., Tora, L., Georgiev, P., and Soldatov, A. (2001). The novel transcription factor e(y)2 interacts with TAF(II)40 and potentiates transcription activation on chromatin templates. Molecular and cellular biology *21*, 5223-5231.

Getts, R.C., and Stamato, T.D. (1994). Absence of a Ku-like DNA end binding activity in the xrs double-strand DNA repair-deficient mutant. The Journal of biological chemistry *269*, 15981-15984.

Giannattasio, M., Lazzaro, F., Plevani, P., and Muzi-Falconi, M. (2005). The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6-Bre1 and H3 methylation by Dot1. The Journal of biological chemistry *280*, 9879-9886.

Gonzalez-Aguilera, C., Tous, C., Gomez-Gonzalez, B., Huertas, P., Luna, R., and Aguilera, A. (2008). The THP1-SAC3-SUS1-CDC31 complex works in transcription elongation-mRNA export preventing RNA-mediated genome instability. Molecular biology of the cell *19*, 4310-4318.

Goodarzi, A.A., Jonnalagadda, J.C., Douglas, P., Young, D., Ye, R., Moorhead, G.B., Lees-Miller, S.P., and Khanna, K.K. (2004). Autophosphorylation of ataxia-telangiectasia mutated is regulated by protein phosphatase 2A. The EMBO journal *23*, 4451-4461.

Gottlieb, T.M., and Jackson, S.P. (1993). The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell *72*, 131-142.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F.*, et al.* (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes & development *11*, 1640-1650.

Gravel, S., Chapman, J.R., Magill, C., and Jackson, S.P. (2008). DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes & development *22*, 2767-2772.

Grewal, S.I., and Jia, S. (2007). Heterochromatin revisited. Nature reviews Genetics *8*, 35-46.

Gu, J., Lu, H., Tippin, B., Shimazaki, N., Goodman, M.F., and Lieber, M.R. (2007a). XRCC4:DNA ligase IV can ligate incompatible DNA ends and can ligate across gaps. The EMBO journal *26*, 1010-1023.

Gu, J., Lu, H., Tsai, A.G., Schwarz, K., and Lieber, M.R. (2007b). Single-stranded DNA ligation and XLF-stimulated incompatible DNA end ligation by the XRCC4-DNA ligase IV complex: influence of terminal DNA sequence. Nucleic acids research *35*, 5755-5762.

Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de Klein, A., Wessels, L., de Laat, W.*, et al.* (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature *453*, 948-951.

Haince, J.F., McDonald, D., Rodrigue, A., Dery, U., Masson, J.Y., Hendzel, M.J., and Poirier, G.G. (2008). PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. The Journal of biological chemistry *283*, 1197-1208.

Hakme, A., Wong, H.K., Dantzer, F., and Schreiber, V. (2008). The expanding field of poly(ADP-ribosyl)ation reactions. 'Protein Modifications: Beyond the Usual Suspects' Review Series. EMBO reports *9*, 1094-1100.

Hase, M.E., and Cordes, V.C. (2003). Direct interaction with nup153 mediates binding of Tpr to the periphery of the nuclear pore complex. Molecular biology of the cell *14*, 1923-1940.

Helmlinger, D., and Tora, L. (2017). Sharing the SAGA. Trends in biochemical sciences.

Helmrich, A., Ballarino, M., Nudler, E., and Tora, L. (2013). Transcription-replication encounters, consequences and genomic instability. Nature structural & molecular biology *20*, 412-418.

Henry, K.W., Wyce, A., Lo, W.S., Duggan, L.J., Emre, N.C., Kao, C.F., Pillus, L., Shilatifard, A., Osley, M.A., and Berger, S.L. (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes & development *17*, 2648-2663.

Hershko, A., and Ciechanover, A. (1998). The ubiquitin system. Annual review of biochemistry *67*, 425-479.

Heyer, W.D., Li, X., Rolfsmeier, M., and Zhang, X.P. (2006). Rad54: the Swiss Army knife of homologous recombination? Nucleic acids research *34*, 4115-4125.

Hoelz, A., Debler, E.W., and Blobel, G. (2011). The structure of the nuclear pore complex. Annual review of biochemistry *80*, 613-643.

Hopfner, K.P., Craig, L., Moncalian, G., Zinkel, R.A., Usui, T., Owen, B.A., Karcher, A., Henderson, B., Bodmer, J.L., McMurray, C.T.*, et al.* (2002). The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature *418*, 562-566.

Hopfner, K.P., Karcher, A., Craig, L., Woo, T.T., Carney, J.P., and Tainer, J.A. (2001). Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell *105*, 473-485.

Horigome, C., Okada, T., Shimazu, K., Gasser, S.M., and Mizuta, K. (2011). Ribosome biogenesis factors bind a nuclear envelope SUN domain protein to cluster yeast telomeres. The EMBO journal *30*, 3799-3811.

Horigome, C., Oma, Y., Konishi, T., Schmid, R., Marcomini, I., Hauer, M.H., Dion, V., Harata, M., and Gasser, S.M. (2014). SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. Molecular cell *55*, 626-639.

Hu, Y., Raynard, S., Sehorn, M.G., Lu, X., Bussen, W., Zheng, L., Stark, J.M., Barnes, E.L., Chi, P., Janscak, P.*, et al.* (2007). RECQL5/Recql5 helicase regulates homologous recombination and suppresses tumor formation via disruption of Rad51 presynaptic filaments. Genes & development *21*, 3073-3084.

Hu, Y., Scully, R., Sobhian, B., Xie, A., Shestakova, E., and Livingston, D.M. (2011). RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. Genes & development *25*, 685-700.

Huen, M.S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M.B., and Chen, J. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell *131*, 901-914.

Huen, M.S., Sy, S.M., and Chen, J. (2010). BRCA1 and its toolbox for the maintenance of genome integrity. Nature reviews Molecular cell biology *11*, 138-148.

Huertas, P., Cortes-Ledesma, F., Sartori, A.A., Aguilera, A., and Jackson, S.P. (2008). CDK targets Sae2 to control DNA-end resection and homologous recombination. Nature *455*, 689-692.

Huertas, P., and Jackson, S.P. (2009). Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. The Journal of biological chemistry *284*, 9558-9565.

Huisinga, K.L., and Pugh, B.F. (2004). A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in Saccharomyces cerevisiae. Molecular cell *13*, 573-585.

Hwang, W.W., Venkatasubrahmanyam, S., Ianculescu, A.G., Tong, A., Boone, C., and Madhani, H.D. (2003). A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. Molecular cell *11*, 261-266.

Ira, G., Pellicioli, A., Balijja, A., Wang, X., Fiorani, S., Carotenuto, W., Liberi, G., Bressan, D., Wan, L., Hollingsworth, N.M.*, et al.* (2004). DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature *431*, 1011-1017.

Isono, M., Niimi, A., Oike, T., Hagiwara, Y., Sato, H., Sekine, R., Yoshida, Y., Isobe, S.Y., Obuse, C., Nishi, R.*, et al.* (2017). BRCA1 Directs the Repair Pathway to Homologous Recombination by Promoting 53BP1 Dephosphorylation. Cell reports *18*, 520-532.

Iwabuchi, K., Bartel, P.L., Li, B., Marraccino, R., and Fields, S. (1994). Two cellular proteins that bind to wild-type but not mutant p53. Proceedings of the National Academy of Sciences of the United States of America *91*, 6098-6102.

Jakob, B., Splinter, J., Conrad, S., Voss, K.O., Zink, D., Durante, M., Lobrich, M., and Taucher-Scholz, G. (2011). DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. Nucleic acids research *39*, 6489-6499.

Jani, D., Lutz, S., Hurt, E., Laskey, R.A., Stewart, M., and Wickramasinghe, V.O. (2012). Functional and structural characterization of the mammalian TREX-2 complex that links transcription with nuclear messenger RNA export. Nucleic acids research *40*, 4562-4573.

Jani, D., Lutz, S., Marshall, N.J., Fischer, T., Kohler, A., Ellisdon, A.M., Hurt, E., and Stewart, M. (2009). Sus1, Cdc31, and the Sac3 CID region form a conserved interaction platform that promotes nuclear pore association and mRNA export. Molecular cell *33*, 727-737.

Jazayeri, A., Falck, J., Lukas, C., Bartek, J., Smith, G.C., Lukas, J., and Jackson, S.P. (2006). ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. Nature cell biology *8*, 37-45.

Jones, A.L., Quimby, B.B., Hood, J.K., Ferrigno, P., Keshava, P.H., Silver, P.A., and Corbett, A.H. (2000). SAC3 may link nuclear protein export to cell cycle progression. Proceedings of the National Academy of Sciences of the United States of America *97*, 3224-3229.

Kakarougkas, A., Ismail, A., Katsuki, Y., Freire, R., Shibata, A., and Jeggo, P.A. (2013). Co-operation of BRCA1 and POH1 relieves the barriers posed by 53BP1 and RAP80 to resection. Nucleic acids research *41*, 10298-10311.

Kalb, R., Mallery, D.L., Larkin, C., Huang, J.T., and Hiom, K. (2014). BRCA1 is a histone-H2A-specific ubiquitin ligase. Cell reports *8*, 999-1005.

Kalocsay, M., Hiller, N.J., and Jentsch, S. (2009). Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Molecular cell *33*, 335-343.

Kalverda, B., Pickersgill, H., Shloma, V.V., and Fornerod, M. (2010). Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. Cell *140*, 360-371.

Kanno, S., Kuzuoka, H., Sasao, S., Hong, Z., Lan, L., Nakajima, S., and Yasui, A. (2007). A novel human AP endonuclease with conserved zinc-finger-like motifs involved in DNA strand break responses. The EMBO journal *26*, 2094-2103.

Kantake, N., Sugiyama, T., Kolodner, R.D., and Kowalczykowski, S.C. (2003). The recombination-deficient mutant RPA (rfa1-t11) is displaced slowly from single-stranded DNA by Rad51 protein. The Journal of biological chemistry *278*, 23410-23417.

Karran, P., and Brem, R. (2016). Protein oxidation, UVA and human DNA repair. DNA repair *44*, 178-185.

Kato, K., Nakajima, K., Ui, A., Muto-Terao, Y., Ogiwara, H., and Nakada, S. (2014). Fine-tuning of DNA damage-dependent ubiquitination by OTUB2 supports the DNA repair pathway choice. Molecular cell *53*, 617-630.

Kind, J., and van Steensel, B. (2014). Stochastic genome-nuclear lamina interactions: modulating roles of Lamin A and BAF. Nucleus *5*, 124-130.

Kinner, A., Wu, W., Staudt, C., and Iliakis, G. (2008). Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. Nucleic acids research *36*, 5678-5694.

Kohler, A., and Hurt, E. (2007). Exporting RNA from the nucleus to the cytoplasm. Nature reviews Molecular cell biology *8*, 761-773.

Kohler, A., Schneider, M., Cabal, G.G., Nehrbass, U., and Hurt, E. (2008). Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export. Nature cell biology *10*, 707-715.

Kohler, A., Zimmerman, E., Schneider, M., Hurt, E., and Zheng, N. (2010). Structural basis for assembly and activation of the heterotetrameric SAGA histone H2B deubiquitinase module. Cell *141*, 606-617.

Kolas, N.K., Chapman, J.R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F.D., Panier, S., Mendez, M., Wildenhain, J., Thomson, T.M.*, et al.* (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science *318*, 1637-1640.

Kopytova, D., Popova, V., Kurshakova, M., Shidlovskii, Y., Nabirochkina, E., Brechalov, A., Georgiev, G., and Georgieva, S. (2016). ORC interacts with THSC/TREX-2 and its subunits promote Nxf1 association with mRNP and mRNA export in Drosophila. Nucleic acids research *44*, 4920-4933.

Kozlov, S.V., Graham, M.E., Peng, C., Chen, P., Robinson, P.J., and Lavin, M.F. (2006). Involvement of novel autophosphorylation sites in ATM activation. The EMBO journal *25*, 3504-3514.

Kraus, E., Leung, W.Y., and Haber, J.E. (2001). Break-induced replication: a review and an example in budding yeast. Proceedings of the National Academy of Sciences of the United States of America *98*, 8255-8262.

Kurosawa, A., Koyama, H., Takayama, S., Miki, K., Ayusawa, D., Fujii, M., Iiizumi, S., and Adachi, N. (2008). The requirement of Artemis in double-strand break repair depends on the type of DNA damage. DNA and cell biology *27*, 55-61.

Kuwahara, K., Fujimura, S., Takahashi, Y., Nakagata, N., Takemori, T., Aizawa, S., and Sakaguchi, N. (2004). Germinal center-associated nuclear protein contributes to affinity maturation of B cell antigen receptor in T cell-dependent responses. Proceedings of the National Academy of Sciences of the United States of America *101*, 1010-1015.

Kuwahara, K., Tomiyasu, S., Fujimura, S., Nomura, K., Xing, Y., Nishiyama, N., Ogawa, M., Imajoh-Ohmi, S., Izuta, S., and Sakaguchi, N. (2001). Germinal center-associated nuclear protein (GANP) has a phosphorylation-dependent DNA-primase activity that is up-regulated in germinal center regions. Proceedings of the National Academy of Sciences of the United States of America *98*, 10279-10283.

Kuwahara, K., Yamamoto-Ibusuki, M., Zhang, Z., Phimsen, S., Gondo, N., Yamashita, H., Takeo, T., Nakagata, N., Yamashita, D., Fukushima, Y.*, et al.* (2016). GANP protein encoded on human chromosome 21/mouse chromosome 10 is associated with resistance to mammary tumor development. Cancer science *107*, 469-477.

Kuwahara, K., Yoshida, M., Kondo, E., Sakata, A., Watanabe, Y., Abe, E., Kouno, Y., Tomiyasu, S., Fujimura, S., Tokuhisa, T.*, et al.* (2000). A novel nuclear phosphoprotein, GANP, is up-regulated in centrocytes of the germinal center and associated with MCM3, a protein essential for DNA replication. Blood *95*, 2321-2328.

Lavin, M.F. (2007). ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. Oncogene *26*, 7749-7758.

Lavin, M.F. (2008). Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. Nature reviews Molecular cell biology *9*, 759-769.

Lavin, M.F., and Kozlov, S. (2007). ATM activation and DNA damage response. Cell cycle *6*, 931-942.

Lee, J.H., and Paull, T.T. (2005). ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science *308*, 551-554.

Lee, J.H., and Paull, T.T. (2007). Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. Oncogene *26*, 7741-7748.

Lee, K.J., Saha, J., Sun, J., Fattah, K.R., Wang, S.C., Jakob, B., Chi, L., Wang, S.Y., Taucher-Scholz, G., Davis, A.J.*, et al.* (2016). Phosphorylation of Ku dictates DNA

double-strand break (DSB) repair pathway choice in S phase. Nucleic acids research *44*, 1732-1745.

Lee, K.K., Sardiu, M.E., Swanson, S.K., Gilmore, J.M., Torok, M., Grant, P.A., Florens, L., Workman, J.L., and Washburn, M.P. (2011). Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes. Molecular systems biology *7*, 503.

Lee, S.E., Mitchell, R.A., Cheng, A., and Hendrickson, E.A. (1997). Evidence for DNA-PK-dependent and -independent DNA double-strand break repair pathways in mammalian cells as a function of the cell cycle. Molecular and cellular biology *17*, 1425-1433.

Lee, T.I., Causton, H.C., Holstege, F.C., Shen, W.C., Hannett, N., Jennings, E.G., Winston, F., Green, M.R., and Young, R.A. (2000). Redundant roles for the TFIID and SAGA complexes in global transcription. Nature *405*, 701-704.

Lee-Theilen, M., Matthews, A.J., Kelly, D., Zheng, S., and Chaudhuri, J. (2011). CtIP promotes microhomology-mediated alternative end joining during class-switch recombination. Nature structural & molecular biology *18*, 75-79.

Lelek, M., Casartelli, N., Pellin, D., Rizzi, E., Souque, P., Severgnini, M., Di Serio, C., Fricke, T., Diaz-Griffero, F., Zimmer, C.*, et al.* (2015). Chromatin organization at the nuclear pore favours HIV replication. Nature communications *6*, 6483.

Lemaitre, C., Fischer, B., Kalousi, A., Hoffbeck, A.S., Guirouilh-Barbat, J., Shahar, O.D., Genet, D., Goldberg, M., Betrand, P., Lopez, B.*, et al.* (2012). The nucleoporin 153, a novel factor in double-strand break repair and DNA damage response. Oncogene *31*, 4803-4809.

Lemaitre, C., Grabarz, A., Tsouroula, K., Andronov, L., Furst, A., Pankotai, T., Heyer, V., Rogier, M., Attwood, K.M., Kessler, P.*, et al.* (2014). Nuclear position dictates DNA repair pathway choice. Genes & development *28*, 2450-2463.

Li, W., Atanassov, B.S., Lan, X., Mohan, R.D., Swanson, S.K., Farria, A.T., Florens, L., Washburn, M.P., Workman, J.L., and Dent, S.Y. (2016). Cytoplasmic ATXN7L3B Interferes with Nuclear Functions of the SAGA Deubiquitinase Module. Molecular and cellular biology *36*, 2855-2866.

Li, X., Seidel, C.W., Szerszen, L.T., Lange, J.J., Workman, J.L., and Abmayr, S.M. (2017). Enzymatic modules of the SAGA chromatin-modifying complex play distinct roles in Drosophila gene expression and development. Genes & development.

Liang, L., Deng, L., Nguyen, S.C., Zhao, X., Maulion, C.D., Shao, C., and Tischfield, J.A. (2008). Human DNA ligases I and III, but not ligase IV, are required for microhomology-mediated end joining of DNA double-strand breaks. Nucleic acids research *36*, 3297-3310.

Limbo, O., Moiani, D., Kertokalio, A., Wyman, C., Tainer, J.A., and Russell, P. (2012). Mre11 ATLD17/18 mutation retains Tel1/ATM activity but blocks DNA double-strand break repair. Nucleic acids research *40*, 11435-11449.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. Nature *362*, 709-715.

Lindahl, T., and Barnes, D.E. (2000). Repair of endogenous DNA damage. Cold Spring Harbor symposia on quantitative biology *65*, 127-133.

Liu, D., Keijzers, G., and Rasmussen, L.J. (2017). DNA mismatch repair and its many roles in eukaryotic cells. Mutation research *773*, 174-187.

Llorente, B., Smith, C.E., and Symington, L.S. (2008). Break-induced replication: what is it and what is it for? Cell cycle *7*, 859-864.

Lobrich, M., Shibata, A., Beucher, A., Fisher, A., Ensminger, M., Goodarzi, A.A., Barton, O., and Jeggo, P.A. (2010). gammaH2AX foci analysis for monitoring DNA double-strand break repair: strengths, limitations and optimization. Cell cycle *9*, 662-669.

Loeillet, S., Palancade, B., Cartron, M., Thierry, A., Richard, G.F., Dujon, B., Doye, V., and Nicolas, A. (2005). Genetic network interactions among replication, repair and nuclear pore deficiencies in yeast. DNA repair *4*, 459-468.

Lou, Z., Minter-Dykhouse, K., Franco, S., Gostissa, M., Rivera, M.A., Celeste, A., Manis, J.P., van Deursen, J., Nussenzweig, A., Paull, T.T.*, et al.* (2006). MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. Molecular cell *21*, 187-200.

Lu, Q., Tang, X., Tian, G., Wang, F., Liu, K., Nguyen, V., Kohalmi, S.E., Keller, W.A., Tsang, E.W., Harada, J.J.*, et al.* (2010). Arabidopsis homolog of the yeast TREX-2 mRNA export complex: components and anchoring nucleoporin. The Plant journal : for cell and molecular biology *61*, 259-270.

Luger, K., Dechassa, M.L., and Tremethick, D.J. (2012). New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? Nature reviews Molecular cell biology *13*, 436-447.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997a). Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature *389*, 251-260.

Luger, K., Rechsteiner, T.J., Flaus, A.J., Waye, M.M., and Richmond, T.J. (1997b). Characterization of nucleosome core particles containing histone proteins made in bacteria. Journal of molecular biology *272*, 301-311.

Lukas, C., Melander, F., Stucki, M., Falck, J., Bekker-Jensen, S., Goldberg, M., Lerenthal, Y., Jackson, S.P., Bartek, J., and Lukas, J. (2004). Mdc1 couples DNA double-strand break recognition by Nbs1 with its H2AX-dependent chromatin retention. The EMBO journal *23*, 2674-2683.

Mackay, D.R., Howa, A.C., Werner, T.L., and Ullman, K.S. (2017). Nup153 and Nup50 promote recruitment of 53BP1 to DNA repair foci by antagonizing BRCA1-dependent events. Journal of cell science.

Maeda, K., Singh, S.K., Eda, K., Kitabatake, M., Pham, P., Goodman, M.F., and Sakaguchi, N. (2010). GANP-mediated recruitment of activation-induced cytidine deaminase to cell nuclei and to immunoglobulin variable region DNA. The Journal of biological chemistry *285*, 23945-23953.

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., and Lukas, J. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell *131*, 887-900.

Makharashvili, N., Tubbs, A.T., Yang, S.H., Wang, H., Barton, O., Zhou, Y., Deshpande, R.A., Lee, J.H., Lobrich, M., Sleckman, B.P.*, et al.* (2014). Catalytic and noncatalytic roles of the CtIP endonuclease in double-strand break end resection. Molecular cell *54*, 1022-1033.

Martinez, E., Palhan, V.B., Tjernberg, A., Lymar, E.S., Gamper, A.M., Kundu, T.K., Chait, B.T., and Roeder, R.G. (2001). Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. Molecular and cellular biology *21*, 6782-6795.

Matsuoka, S., Rotman, G., Ogawa, A., Shiloh, Y., Tamai, K., and Elledge, S.J. (2000). Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. Proceedings of the National Academy of Sciences of the United States of America *97*, 10389-10394.

Mattiroli, F., Vissers, J.H., van Dijk, W.J., Ikpa, P., Citterio, E., Vermeulen, W., Marteijn, J.A., and Sixma, T.K. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell *150*, 1182-1195.

Mavragani, I.V., Nikitaki, Z., Souli, M.P., Aziz, A., Nowsheen, S., Aziz, K., Rogakou, E., and Georgakilas, A.G. (2017). Complex DNA Damage: A Route to Radiation-Induced Genomic Instability and Carcinogenesis. Cancers *9*.

McKinnon, P.J. (2012). ATM and the molecular pathogenesis of ataxia telangiectasia. Annual review of pathology *7*, 303-321.

Mimitou, E.P., and Symington, L.S. (2008). Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature *455*, 770-774.

Mimori, T., and Hardin, J.A. (1986). Mechanism of interaction between Ku protein and DNA. The Journal of biological chemistry *261*, 10375-10379.

Morales, J.C., Xia, Z., Lu, T., Aldrich, M.B., Wang, B., Rosales, C., Kellems, R.E., Hittelman, W.N., Elledge, S.J., and Carpenter, P.B. (2003). Role for the BRCA1 C-terminal repeats (BRCT) protein 53BP1 in maintaining genomic stability. The Journal of biological chemistry *278*, 14971-14977.

Morris, J.R., and Solomon, E. (2004). BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. Human molecular genetics *13*, 807-817.

Mosbech, A., Lukas, C., Bekker-Jensen, S., and Mailand, N. (2013). The deubiquitylating enzyme USP44 counteracts the DNA double-strand break response mediated by the RNF8 and RNF168 ubiquitin ligases. The Journal of biological chemistry *288*, 16579-16587.

Moyal, L., Lerenthal, Y., Gana-Weisz, M., Mass, G., So, S., Wang, S.Y., Eppink, B., Chung, Y.M., Shalev, G., Shema, E.*, et al.* (2011). Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. Molecular cell *41*, 529-542.

Moynahan, M.E., Chiu, J.W., Koller, B.H., and Jasin, M. (1999). Brca1 controls homology-directed DNA repair. Molecular cell *4*, 511-518.

Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M.B., Roberts, T.M., Brown, G.W., Varela, E., Hediger, F., Gasser, S.M., and Krogan, N.J. (2008). Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science *322*, 597-602.

Nakamura, K., Kato, A., Kobayashi, J., Yanagihara, H., Sakamoto, S., Oliveira, D.V., Shimada, M., Tauchi, H., Suzuki, H., Tashiro, S.*, et al.* (2011). Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. Molecular cell *41*, 515-528.

Nassif, N., and Engels, W. (1993). DNA homology requirements for mitotic gap repair in Drosophila. Proceedings of the National Academy of Sciences of the United States of America *90*, 1262-1266.

Ng, H.M., Wei, L., Lan, L., and Huen, M.S. (2016). The Lys63-deubiquitylating Enzyme BRCC36 Limits DNA Break Processing and Repair. The Journal of biological chemistry *291*, 16197-16207.

Nishikawa, H., Ooka, S., Sato, K., Arima, K., Okamoto, J., Klevit, R.E., Fukuda, M., and Ohta, T. (2004). Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. The Journal of biological chemistry *279*, 3916-3924.

Orr-Weaver, T.L., Szostak, J.W., and Rothstein, R.J. (1981). Yeast transformation: a model system for the study of recombination. Proceedings of the National Academy of Sciences of the United States of America *78*, 6354-6358.

Oza, P., Jaspersen, S.L., Miele, A., Dekker, J., and Peterson, C.L. (2009). Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes & development *23*, 912-927.

Paddock, M.N., Bauman, A.T., Higdon, R., Kolker, E., Takeda, S., and Scharenberg, A.M. (2011). Competition between PARP-1 and Ku70 control the decision between high-fidelity and mutagenic DNA repair. DNA repair *10*, 338-343.

Paillard, S., and Strauss, F. (1991). Analysis of the mechanism of interaction of simian Ku protein with DNA. Nucleic acids research *19*, 5619-5624.

Palancade, B., Liu, X., Garcia-Rubio, M., Aguilera, A., Zhao, X., and Doye, V. (2007). Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. Molecular biology of the cell *18*, 2912-2923.

Pannunzio, N.R., Li, S., Watanabe, G., and Lieber, M.R. (2014). Non-homologous end joining often uses microhomology: implications for alternative end joining. DNA repair *17*, 74-80.

Paques, F., Leung, W.Y., and Haber, J.E. (1998). Expansions and contractions in a tandem repeat induced by double-strand break repair. Molecular and cellular biology *18*, 2045-2054.

Park, J.M., and Kang, T.H. (2016). Transcriptional and Posttranslational Regulation of Nucleotide Excision Repair: The Guardian of the Genome against Ultraviolet Radiation. International journal of molecular sciences *17*.

Pascual-Garcia, P., Govind, C.K., Queralt, E., Cuenca-Bono, B., Llopis, A., Chavez, S., Hinnebusch, A.G., and Rodriguez-Navarro, S. (2008). Sus1 is recruited to coding regions and functions during transcription elongation in association with SAGA and TREX2. Genes & development *22*, 2811-2822.

Passarge, E. (1979). Emil Heitz and the concept of heterochromatin: longitudinal chromosome differentiation was recognized fifty years ago. American journal of human genetics *31*, 106-115.

Pellegrini, L., Yu, D.S., Lo, T., Anand, S., Lee, M., Blundell, T.L., and Venkitaraman, A.R. (2002). Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. Nature *420*, 287-293.

Perez-Garrastachu, M., Arluzea, J., Andrade, R., Diez-Torre, A., Urtizberea, M., Silio, M., and Arechaga, J. (2017). Nucleoporins redistribute inside the nucleus after cell cycle arrest induced by histone deacetylases inhibition. Nucleus, 1-19.

Peric-Hupkes, D., Meuleman, W., Pagie, L., Bruggeman, S.W., Solovei, I., Brugman, W., Graf, S., Flicek, P., Kerkhoven, R.M., van Lohuizen, M.*, et al.* (2010). Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Molecular cell *38*, 603-613.

Peric-Hupkes, D., and van Steensel, B. (2010). Role of the nuclear lamina in genome organization and gene expression. Cold Spring Harbor symposia on quantitative biology *75*, 517-524.

Peterson, S.R., Kurimasa, A., Oshimura, M., Dynan, W.S., Bradbury, E.M., and Chen, D.J. (1995). Loss of the catalytic subunit of the DNA-dependent protein kinase in DNA double-strand-break-repair mutant mammalian cells. Proceedings of the National Academy of Sciences of the United States of America *92*, 3171-3174.

Petukhova, G., Stratton, S., and Sung, P. (1998). Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature *393*, 91-94.

Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M., and van Steensel, B. (2006). Characterization of the Drosophila melanogaster genome at the nuclear lamina. Nature genetics *38*, 1005-1014.

Pilch, D.R., Sedelnikova, O.A., Redon, C., Celeste, A., Nussenzweig, A., and Bonner, W.M. (2003). Characteristics of gamma-H2AX foci at DNA double-strand breaks sites. Biochemistry and cell biology = Biochimie et biologie cellulaire *81*, 123-129.

Prado, F., and Aguilera, A. (1995). Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the RAD1, RAD10, and RAD52 genes. Genetics *139*, 109-123.

Prado, F., Piruat, J.I., and Aguilera, A. (1997). Recombination between DNA repeats in yeast hpr1delta cells is linked to transcription elongation. The EMBO journal *16*, 2826-2835.

Qi, Z., Redding, S., Lee, J.Y., Gibb, B., Kwon, Y., Niu, H., Gaines, W.A., Sung, P., and Greene, E.C. (2015). DNA sequence alignment by microhomology sampling during homologous recombination. Cell *160*, 856-869.

Rabut, G., Doye, V., and Ellenberg, J. (2004). Mapping the dynamic organization of the nuclear pore complex inside single living cells. Nature cell biology *6*, 1114-1121.

Ragoczy, T., Bender, M.A., Telling, A., Byron, R., and Groudine, M. (2006). The locus control region is required for association of the murine beta-globin locus with engaged transcription factories during erythroid maturation. Genes & development *20*, 1447-1457.

Rahal, E.A., Henricksen, L.A., Li, Y., Williams, R.S., Tainer, J.A., and Dixon, K. (2010). ATM regulates Mre11-dependent DNA end-degradation and microhomology-mediated end joining. Cell cycle *9*, 2866-2877.

Ramachandran, S., Haddad, D., Li, C., Le, M.X., Ling, A.K., So, C.C., Nepal, R.M., Gommerman, J.L., Yu, K., Ketela, T.*, et al.* (2016). The SAGA Deubiquitination Module Promotes DNA Repair and Class Switch Recombination through ATM and DNAPK-Mediated gammaH2AX Formation. Cell reports *15*, 1554-1565.

Resnick, M.A. (1976). The repair of double-strand breaks in DNA; a model involving recombination. Journal of theoretical biology *59*, 97-106.

Riballo, E., Kuhne, M., Rief, N., Doherty, A., Smith, G.C., Recio, M.J., Reis, C., Dahm, K., Fricke, A., Krempler, A.*, et al.* (2004). A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. Molecular cell *16*, 715-724.

Robert, I., Dantzer, F., and Reina-San-Martin, B. (2009). Parp1 facilitates alternative NHEJ, whereas Parp2 suppresses IgH/c-myc translocations during immunoglobulin class switch recombination. The Journal of experimental medicine *206*, 1047-1056.

Robzyk, K., Recht, J., and Osley, M.A. (2000). Rad6-dependent ubiquitination of histone H2B in yeast. Science *287*, 501-504.

Rodriguez-Navarro, S., Fischer, T., Luo, M.J., Antunez, O., Brettschneider, S., Lechner, J., Perez-Ortin, J.E., Reed, R., and Hurt, E. (2004). Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. Cell *116*, 75-86.

Rogakou, E.P., Boon, C., Redon, C., and Bonner, W.M. (1999). Megabase chromatin domains involved in DNA double-strand breaks in vivo. The Journal of cell biology *146*, 905-916.

Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., and Bonner, W.M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. The Journal of biological chemistry *273*, 5858-5868.

Rohner, S., Kalck, V., Wang, X., Ikegami, K., Lieb, J.D., Gasser, S.M., and Meister, P. (2013). Promoter- and RNA polymerase II-dependent hsp-16 gene association with nuclear pores in Caenorhabditis elegans. The Journal of cell biology *200*, 589-604.

Rondon, A.G., Garcia-Rubio, M., Gonzalez-Barrera, S., and Aguilera, A. (2003). Molecular evidence for a positive role of Spt4 in transcription elongation. The EMBO journal *22*, 612-620.

Roukos, V., Voss, T.C., Schmidt, C.K., Lee, S., Wangsa, D., and Misteli, T. (2013). Spatial dynamics of chromosome translocations in living cells. Science *341*, 660-664.

Ryu, T., Spatola, B., Delabaere, L., Bowlin, K., Hopp, H., Kunitake, R., Karpen, G.H., and Chiolo, I. (2015). Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. Nature cell biology *17*, 1401-1411.

Saito, S., Goodarzi, A.A., Higashimoto, Y., Noda, Y., Lees-Miller, S.P., Appella, E., and Anderson, C.W. (2002). ATM mediates phosphorylation at multiple p53 sites, including Ser(46), in response to ionizing radiation. The Journal of biological chemistry *277*, 12491-12494.

Sakaguchi, N., and Maeda, K. (2016). Germinal Center B-Cell-Associated Nuclear Protein (GANP) Involved in RNA Metabolism for B Cell Maturation. Advances in immunology *131*, 135-186.

Sakaguchi, N., Maeda, K., and Kuwahara, K. (2011). Molecular mechanism of immunoglobulin V-region diversification regulated by transcription and RNA metabolism in antigen-driven B cells. Scandinavian journal of immunology *73*, 520-526.

Samara, N.L., Datta, A.B., Berndsen, C.E., Zhang, X., Yao, T., Cohen, R.E., and Wolberger, C. (2010). Structural insights into the assembly and function of the SAGA deubiquitinating module. Science *328*, 1025-1029.

San Filippo, J., Sung, P., and Klein, H. (2008). Mechanism of eukaryotic homologous recombination. Annual review of biochemistry *77*, 229-257.

Sanders, S.L., Jennings, J., Canutescu, A., Link, A.J., and Weil, P.A. (2002). Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry. Molecular and cellular biology *22*, 4723-4738.

Savage, K.I., and Harkin, D.P. (2015). BRCA1, a 'complex' protein involved in the maintenance of genomic stability. The FEBS journal *282*, 630-646.

Scharer, O.D. (2013). Nucleotide excision repair in eukaryotes. Cold Spring Harbor perspectives in biology *5*, a012609.

Schiller, C.B., Lammens, K., Guerini, I., Coordes, B., Feldmann, H., Schlauderer, F., Mockel, C., Schele, A., Strasser, K., Jackson, S.P.*, et al.* (2012). Structure of Mre11-Nbs1 complex yields insights into ataxia-telangiectasia-like disease mutations and DNA damage signaling. Nature structural & molecular biology *19*, 693-700.

Schlegel, B.P., Jodelka, F.M., and Nunez, R. (2006). BRCA1 promotes induction of ssDNA by ionizing radiation. Cancer research *66*, 5181-5189.

Schultz, L.B., Chehab, N.H., Malikzay, A., and Halazonetis, T.D. (2000). p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. The Journal of cell biology *151*, 1381-1390.

Schuurs-Hoeijmakers, J.H., Vulto-van Silfhout, A.T., Vissers, L.E., van de, V., II, van Bon, B.W., de Ligt, J., Gilissen, C., Hehir-Kwa, J.Y., Neveling, K., del Rosario, M.*, et al.* (2013). Identification of pathogenic gene variants in small families with intellectually disabled siblings by exome sequencing. Journal of medical genetics *50*, 802-811.

Schwartz, E.K., and Heyer, W.D. (2011). Processing of joint molecule intermediates by structure-selective endonucleases during homologous recombination in eukaryotes. Chromosoma *120*, 109-127.

Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D.M. (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell *88*, 265-275.

Shang, Y.L., Bodero, A.J., and Chen, P.L. (2003). NFBD1, a novel nuclear protein with signature motifs of FHA and BRCT, and an internal 41-amino acid repeat sequence, is an early participant in DNA damage response. The Journal of biological chemistry *278*, 6323-6329.

Shao, Z., Davis, A.J., Fattah, K.R., So, S., Sun, J., Lee, K.J., Harrison, L., Yang, J., and Chen, D.J. (2012). Persistently bound Ku at DNA ends attenuates DNA end resection and homologous recombination. DNA repair *11*, 310-316.

Sharma, N., Zhu, Q., Wani, G., He, J., Wang, Q.E., and Wani, A.A. (2014). USP3 counteracts RNF168 via deubiquitinating H2A and gammaH2AX at lysine 13 and 15. Cell cycle *13*, 106-114.

Shi, W., Feng, Z., Zhang, J., Gonzalez-Suarez, I., Vanderwaal, R.P., Wu, X., Powell, S.N., Roti Roti, J.L., Gonzalo, S., and Zhang, J. (2010). The role of RPA2 phosphorylation in homologous recombination in response to replication arrest. Carcinogenesis *31*, 994-1002.

Shi, Y., and Whetstine, J.R. (2007). Dynamic regulation of histone lysine methylation by demethylases. Molecular cell *25*, 1-14.

Shibata, A., Conrad, S., Birraux, J., Geuting, V., Barton, O., Ismail, A., Kakarougkas, A., Meek, K., Taucher-Scholz, G., Lobrich, M.*, et al.* (2011). Factors determining DNA double-strand break repair pathway choice in G2 phase. The EMBO journal *30*, 1079-1092.

Shibata, A., Moiani, D., Arvai, A.S., Perry, J., Harding, S.M., Genois, M.M., Maity, R., van Rossum-Fikkert, S., Kertokalio, A., Romoli, F.*, et al.* (2014). DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. Molecular cell *53*, 7-18.

Shivji, M.K., Mukund, S.R., Rajendra, E., Chen, S., Short, J.M., Savill, J., Klenerman, D., and Venkitaraman, A.R. (2009). The BRC repeats of human BRCA2 differentially regulate RAD51 binding on single- versus double-stranded DNA to stimulate strand exchange. Proceedings of the National Academy of Sciences of the United States of America *106*, 13254-13259.

Singh, S.K., Maeda, K., Eid, M.M., Almofty, S.A., Ono, M., Pham, P., Goodman, M.F., and Sakaguchi, N. (2013). GANP regulates recruitment of AID to immunoglobulin variable regions by modulating transcription and nucleosome occupancy. Nature communications *4*, 1830.

Sobhian, B., Shao, G., Lilli, D.R., Culhane, A.C., Moreau, L.A., Xia, B., Livingston, D.M., and Greenberg, R.A. (2007). RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science *316*, 1198-1202.

Sommers, J.A., Rawtani, N., Gupta, R., Bugreev, D.V., Mazin, A.V., Cantor, S.B., and Brosh, R.M., Jr. (2009). FANCJ uses its motor ATPase to destabilize protein-DNA complexes, unwind triplexes, and inhibit RAD51 strand exchange. The Journal of biological chemistry *284*, 7505-7517.

Soutoglou, E., Dorn, J.F., Sengupta, K., Jasin, M., Nussenzweig, A., Ried, T., Danuser, G., and Misteli, T. (2007). Positional stability of single double-strand breaks in mammalian cells. Nature cell biology *9*, 675-682.

Starita, L.M., and Parvin, J.D. (2006). Substrates of the BRCA1-dependent ubiquitin ligase. Cancer biology & therapy *5*, 137-141.

Stewart, G.S., Panier, S., Townsend, K., Al-Hakim, A.K., Kolas, N.K., Miller, E.S., Nakada, S., Ylanko, J., Olivarius, S., Mendez, M.*, et al.* (2009). The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. Cell *136*, 420-434.

Stewart, G.S., Wang, B., Bignell, C.R., Taylor, A.M., and Elledge, S.J. (2003). MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature *421*, 961-966.

Stiff, T., O'Driscoll, M., Rief, N., Iwabuchi, K., Lobrich, M., and Jeggo, P.A. (2004). ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. Cancer research *64*, 2390-2396.

Strauss, C., and Goldberg, M. (2011). Recruitment of proteins to DNA double-strand breaks: MDC1 directly recruits RAP80. Cell cycle *10*, 2850-2857.

Stucki, M. (2009). Histone H2A.X Tyr142 phosphorylation: a novel sWItCH for apoptosis? DNA repair *8*, 873-876.

Stucki, M., Clapperton, J.A., Mohammad, D., Yaffe, M.B., Smerdon, S.J., and Jackson, S.P. (2005). MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell *123*, 1213-1226.

Stucki, M., and Jackson, S.P. (2006). gammaH2AX and MDC1: anchoring the DNA-damage-response machinery to broken chromosomes. DNA repair *5*, 534-543.

Sun, J., Lee, K.J., Davis, A.J., and Chen, D.J. (2012). Human Ku70/80 protein blocks exonuclease 1-mediated DNA resection in the presence of human Mre11 or Mre11/Rad50 protein complex. The Journal of biological chemistry *287*, 4936-4945.

Sun, Y., Jiang, X., Chen, S., Fernandes, N., and Price, B.D. (2005). A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proceedings of the National Academy of Sciences of the United States of America *102*, 13182-13187.

Sun, Y., Jiang, X., Xu, Y., Ayrapetov, M.K., Moreau, L.A., Whetstine, J.R., and Price, B.D. (2009). Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. Nature cell biology *11*, 1376-1382.

Sun, Y., Xu, Y., Roy, K., and Price, B.D. (2007). DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. Molecular and cellular biology *27*, 8502-8509.

Sung, P. (1997). Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. The Journal of biological chemistry *272*, 28194-28197.

Sung, P., Krejci, L., Van Komen, S., and Sehorn, M.G. (2003). Rad51 recombinase and recombination mediators. The Journal of biological chemistry *278*, 42729-42732.

Suwa, A., Hirakata, M., Takeda, Y., Jesch, S.A., Mimori, T., and Hardin, J.A. (1994). DNA-dependent protein kinase (Ku protein-p350 complex) assembles on double-stranded DNA. Proceedings of the National Academy of Sciences of the United States of America *91*, 6904-6908.

Sy, S.M., Huen, M.S., and Chen, J. (2009). PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proceedings of the National Academy of Sciences of the United States of America *106*, 7155-7160.

Symington, L.S., Rothstein, R., and Lisby, M. (2014). Mechanisms and regulation of mitotic recombination in Saccharomyces cerevisiae. Genetics *198*, 795-835.

Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., and Stahl, F.W. (1983). The double-strand-break repair model for recombination. Cell *33*, 25-35.

Taccioli, G.E., Gottlieb, T.M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A.R., Alt, F.W., Jackson, S.P., and Jeggo, P.A. (1994). Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. Science *265*, 1442-1445.

Takahashi, A., and Ohnishi, T. (2005). Does gammaH2AX foci formation depend on the presence of DNA double strand breaks? Cancer letters *229*, 171-179.

Takata, M., Sasaki, M.S., Tachiiri, S., Fukushima, T., Sonoda, E., Schild, D., Thompson, L.H., and Takeda, S. (2001). Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. Molecular and cellular biology *21*, 2858-2866.

Takei, Y., Swietlik, M., Tanoue, A., Tsujimoto, G., Kouzarides, T., and Laskey, R. (2001). MCM3AP, a novel acetyltransferase that acetylates replication protein MCM3. EMBO reports *2*, 119-123.

Takei, Y., and Tsujimoto, G. (1998). Identification of a novel MCM3-associated protein that facilitates MCM3 nuclear localization. The Journal of biological chemistry *273*, 22177-22180.

Takizawa, T., Gudla, P.R., Guo, L., Lockett, S., and Misteli, T. (2008). Allele-specific nuclear positioning of the monoallelically expressed astrocyte marker GFAP. Genes & development *22*, 489-498.

Tang, J., Cho, N.W., Cui, G., Manion, E.M., Shanbhag, N.M., Botuyan, M.V., Mer, G., and Greenberg, R.A. (2013). Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. Nature structural & molecular biology *20*, 317-325.

Tartier, L., Spenlehauer, C., Newman, H.C., Folkard, M., Prise, K.M., Michael, B.D., Menissier-de Murcia, J., and de Murcia, G. (2003). Local DNA damage by proton microbeam irradiation induces poly(ADP-ribose) synthesis in mammalian cells. Mutagenesis *18*, 411-416.

Techer, H., Koundrioukoff, S., Nicolas, A., and Debatisse, M. (2017). The impact of replication stress on replication dynamics and DNA damage in vertebrate cells. Nature reviews Genetics *18*, 535-550.

Thacker, J. (2005). The RAD51 gene family, genetic instability and cancer. Cancer letters *219*, 125-135.

Thompson, L.H. (2012). Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutation research *751*, 158-246.

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., Narita, T., Sixma, T.K., Choudhary, C., Bekker-Jensen, S.*, et al.* (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature *527*, 389-393.

Thorslund, T., and West, S.C. (2007). BRCA2: a universal recombinase regulator. Oncogene *26*, 7720-7730.

Tirosh, I., and Barkai, N. (2008). Two strategies for gene regulation by promoter nucleosomes. Genome research *18*, 1084-1091.

Torres-Rosell, J., Sunjevaric, I., De Piccoli, G., Sacher, M., Eckert-Boulet, N., Reid, R., Jentsch, S., Rothstein, R., Aragon, L., and Lisby, M. (2007). The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. Nature cell biology *9*, 923-931.

Truong, L.N., Li, Y., Shi, L.Z., Hwang, P.Y., He, J., Wang, H., Razavian, N., Berns, M.W., and Wu, X. (2013). Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America *110*, 7720-7725.

Tsai, C.J., Kim, S.A., and Chu, G. (2007). Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends. Proceedings of the National Academy of Sciences of the United States of America *104*, 7851-7856.

Tsouroula, K., Furst, A., Rogier, M., Heyer, V., Maglott-Roth, A., Ferrand, A., Reina-San-Martin, B., and Soutoglou, E. (2016). Temporal and Spatial Uncoupling of DNA Double Strand Break Repair Pathways within Mammalian Heterochromatin. Molecular cell *63*, 293-305.

Typas, D., Luijsterburg, M.S., Wiegant, W.W., Diakatou, M., Helfricht, A., Thijssen, P.E., van den Broek, B., Mullenders, L.H., and van Attikum, H. (2015). The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. Nucleic acids research *43*, 6919-6933.

Umlauf, D., Bonnet, J., Waharte, F., Fournier, M., Stierle, M., Fischer, B., Brino, L., Devys, D., and Tora, L. (2013). The human TREX-2 complex is stably associated with the nuclear pore basket. Journal of cell science *126*, 2656-2667.

Vassilev, L.T., Tovar, C., Chen, S., Knezevic, D., Zhao, X., Sun, H., Heimbrook, D.C., and Chen, L. (2006). Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1. Proceedings of the National Academy of Sciences of the United States of America *103*, 10660-10665.

Venters, B.J., Wachi, S., Mavrich, T.N., Andersen, B.E., Jena, P., Sinnamon, A.J., Jain, P., Rolleri, N.S., Jiang, C., Hemeryck-Walsh, C.*, et al.* (2011). A comprehensive genomic binding map of gene and chromatin regulatory proteins in Saccharomyces. Molecular cell *41*, 480-492.

Wallace, S.S. (2014). Base excision repair: a critical player in many games. DNA repair *19*, 14-26.

Wang, H., Perrault, A.R., Takeda, Y., Qin, W., Wang, H., and Iliakis, G. (2003). Biochemical evidence for Ku-independent backup pathways of NHEJ. Nucleic acids research *31*, 5377-5388.

Wang, H., Rosidi, B., Perrault, R., Wang, M., Zhang, L., Windhofer, F., and Iliakis, G. (2005). DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. Cancer research *65*, 4020-4030.

Wang, M., Wu, W., Wu, W., Rosidi, B., Zhang, L., Wang, H., and Iliakis, G. (2006). PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic acids research *34*, 6170-6182.

Wang, Z., Zhang, H., Liu, J., Cheruiyot, A., Lee, J.H., Ordog, T., Lou, Z., You, Z., and Zhang, Z. (2016). USP51 deubiquitylates H2AK13,15ub and regulates DNA damage response. Genes & development *30*, 946-959.

Ward, I.M., Minn, K., van Deursen, J., and Chen, J. (2003). p53 Binding protein 53BP1 is required for DNA damage responses and tumor suppression in mice. Molecular and cellular biology *23*, 2556-2563.

Watson, M.L. (1955). The nuclear envelope; its structure and relation to cytoplasmic membranes. The Journal of biophysical and biochemical cytology *1*, 257-270.

West, M.H., and Bonner, W.M. (1980). Histone 2B can be modified by the attachment of ubiquitin. Nucleic acids research *8*, 4671-4680.

Wickramasinghe, V.O., Andrews, R., Ellis, P., Langford, C., Gurdon, J.B., Stewart, M., Venkitaraman, A.R., and Laskey, R.A. (2014). Selective nuclear export of specific classes of mRNA from mammalian nuclei is promoted by GANP. Nucleic acids research *42*, 5059-5071.

Wickramasinghe, V.O., McMurtrie, P.I., Marr, J., Amagase, Y., Main, S., Mills, A.D., Laskey, R.A., and Takei, Y. (2011). MCM3AP is transcribed from a promoter within an intron of the overlapping gene for GANP. Journal of molecular biology *406*, 355-361.

Wickramasinghe, V.O., McMurtrie, P.I., Mills, A.D., Takei, Y., Penrhyn-Lowe, S., Amagase, Y., Main, S., Marr, J., Stewart, M., and Laskey, R.A. (2010). mRNA export from mammalian cell nuclei is dependent on GANP. Current biology : CB *20*, 25-31.

Wickramasinghe, V.O., and Venkitaraman, A.R. (2016). RNA Processing and Genome Stability: Cause and Consequence. Molecular cell *61*, 496-505.

Williams, R.R., Azuara, V., Perry, P., Sauer, S., Dvorkina, M., Jorgensen, H., Roix, J., McQueen, P., Misteli, T., Merkenschlager, M.*, et al.* (2006). Neural induction promotes large-scale chromatin reorganisation of the Mash1 locus. Journal of cell science *119*, 132-140.

Wilson, M.D., Benlekbir, S., Fradet-Turcotte, A., Sherker, A., Julien, J.P., McEwan, A., Noordermeer, S.M., Sicheri, F., Rubinstein, J.L., and Durocher, D. (2016). The structural basis of modified nucleosome recognition by 53BP1. Nature *536*, 100-103.

Wolner, B., van Komen, S., Sung, P., and Peterson, C.L. (2003). Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. Molecular cell *12*, 221-232.

Wood, A., Krogan, N.J., Dover, J., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J.F.*, et al.* (2003). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Molecular cell *11*, 267-274.

Wu, D., Topper, L.M., and Wilson, T.E. (2008). Recruitment and dissociation of nonhomologous end joining proteins at a DNA double-strand break in Saccharomyces cerevisiae. Genetics *178*, 1237-1249.

Wu, L.C., Wang, Z.W., Tsan, J.T., Spillman, M.A., Phung, A., Xu, X.L., Yang, M.C., Hwang, L.Y., Bowcock, A.M., and Baer, R. (1996). Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nature genetics *14*, 430-440.

Wu, W., Nishikawa, H., Fukuda, T., Vittal, V., Asano, M., Miyoshi, Y., Klevit, R.E., and Ohta, T. (2015). Interaction of BARD1 and HP1 Is Required for BRCA1 Retention at Sites of DNA Damage. Cancer research *75*, 1311-1321.

Wyatt, D.W., Feng, W., Conlin, M.P., Yousefzadeh, M.J., Roberts, S.A., Mieczkowski, P., Wood, R.D., Gupta, G.P., and Ramsden, D.A. (2016). Essential Roles for Polymerase theta-Mediated End Joining in the Repair of Chromosome Breaks. Molecular cell *63*, 662-673.

Xia, B., Sheng, Q., Nakanishi, K., Ohashi, A., Wu, J., Christ, N., Liu, X., Jasin, M., Couch, F.J., and Livingston, D.M. (2006). Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. Molecular cell *22*, 719-729.

Xiao, A., Li, H., Shechter, D., Ahn, S.H., Fabrizio, L.A., Erdjument-Bromage, H., Ishibe-Murakami, S., Wang, B., Tempst, P., Hofmann, K.*, et al.* (2009). WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. Nature *457*, 57-62.

Xie, A., Kwok, A., and Scully, R. (2009). Role of mammalian Mre11 in classical and alternative nonhomologous end joining. Nature structural & molecular biology *16*, 814-818.

Yang, Y., La, H., Tang, K., Miki, D., Yang, L., Wang, B., Duan, C.G., Nie, W., Wang, X., Wang, S.*, et al.* (2017). SAC3B, a central component of the mRNA export complex TREX-2, is required for prevention of epigenetic gene silencing in Arabidopsis. Nucleic acids research *45*, 181-197.

Yarden, R.I., Pardo-Reoyo, S., Sgagias, M., Cowan, K.H., and Brody, L.C. (2002). BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage. Nature genetics *30*, 285-289.

Yoshida, M., Kuwahara, K., Shimasaki, T., Nakagata, N., Matsuoka, M., and Sakaguchi, N. (2007). GANP suppresses DNA recombination, measured by direct-repeat beta-galactosidase gene construct, but does not suppress the type of recombination applying to immunoglobulin genes in mammalian cells. Genes to cells : devoted to molecular & cellular mechanisms *12*, 1205-1213.

Yu, K., Chedin, F., Hsieh, C.L., Wilson, T.E., and Lieber, M.R. (2003). R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. Nature immunology *4*, 442-451.

Yu, X., and Chen, J. (2004). DNA damage-induced cell cycle checkpoint control requires CtIP, a phosphorylation-dependent binding partner of BRCA1 C-terminal domains. Molecular and cellular biology *24*, 9478-9486.

Yu, X., Wu, L.C., Bowcock, A.M., Aronheim, A., and Baer, R. (1998). The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. The Journal of biological chemistry *273*, 25388-25392.

Yun, M.H., and Hiom, K. (2009). CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature *459*, 460-463.

Zgheib, O., Pataky, K., Brugger, J., and Halazonetis, T.D. (2009). An oligomerized 53BP1 tudor domain suffices for recognition of DNA double-strand breaks. Molecular and cellular biology *29*, 1050-1058.

Zhang, F., Fan, Q., Ren, K., and Andreassen, P.R. (2009a). PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. Molecular cancer research : MCR *7*, 1110-1118.

Zhang, F., Ma, J., Wu, J., Ye, L., Cai, H., Xia, B., and Yu, X. (2009b). PALB2 links BRCA1 and BRCA2 in the DNA-damage response. Current biology : CB *19*, 524-529.

Zhang, X.Y., Varthi, M., Sykes, S.M., Phillips, C., Warzecha, C., Zhu, W., Wyce, A., Thorne, A.W., Berger, S.L., and McMahon, S.B. (2008). The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Molecular cell *29*, 102-111.

Zhang, Y., and Jasin, M. (2011). An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. Nature structural & molecular biology *18*, 80-84.

Zhao, G.Y., Sonoda, E., Barber, L.J., Oka, H., Murakawa, Y., Yamada, K., Ikura, T., Wang, X., Kobayashi, M., Yamamoto, K.*, et al.* (2007). A critical role for the ubiquitin-conjugating enzyme Ubc13 in initiating homologous recombination. Molecular cell *25*, 663-675.

Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., Suzuki, E., Le Guezennec, X., Stunnenberg, H.G., Krasnov, A.*, et al.* (2008). A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Molecular cell *29*, 92-101.

Zhu, B., Zheng, Y., Pham, A.D., Mandal, S.S., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (2005). Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Molecular cell *20*, 601-611.

Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. (2008). Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell *134*, 981-994.

Zou, L., and Elledge, S.J. (2003). Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science *300*, 1542-1548.

UNIVERSITÉ DE STRASBOURG

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erc

Etude du rôle du complexe humain TREX-2 lors de la Réponse aux Dommages de l'ADN

Résumé en Français

L'intégrité de l'information génétique est essentielle aux fonctions cellulaires et pour éviter l'instabilité génomique, qui est une des caractéristiques du cancer. Suite à des cassures double brin (Double Strand Breaks; *DSBs*), la voie de signalisation de réponse aux dommages de l'ADN (DNA Damage Response; *DDR*) est activée dans la cellule. Elle comprend deux sous-voies de signalisation : la jonction d'extrémités non-homologues (Non-Homologous End Joining; *NHEJ*), peu fidèle et encline aux erreurs, et la recombinaison homologue (Homologous Recombination; *HR*), fidèle et non encline aux erreurs. La *HR* est favorisée en phase S et G2 du cycle cellulaire, c'est à dire lorsque la chromatide sœur permet l'utilisation de l'information génomique complémentaire afin de remplacer la portion d'ADN perdue.
Le Complexe TREX-2 associé au Complexe du Pore Nucléaire (Nuclear Pore Complex; *NPC*) est impliqué dans la transcription et l'export des ARNm. Chez la levure, TREX-2 est impliqué dans le maintien de la stabilité génomique à travers le mécanisme de *gene gating*, couplant la transcription et l'export des ARNm au NPC. Ce mécanisme nécessite une connexion entre ces deux processus qui dépend de l'interaction physique et fonctionnelle entre TREX-2 et le complexe co-activateur SAGA.
Le complexe TREX-2 étant conservé à travers l'évolution entre la levure et l'Homme, cela suggère qu'il pourrait aussi avoir un rôle dans le maintien de la stabilité génomique dans les cellules humaines.
Nous nous sommes intéressés au rôle de TREX-2 dans la réparation des *DSBs* dans les cellules humaines. La déplétion de la sous-unité architecturale GANP du complexe TREX-2 entraine une réparation de l'ADN par recombinaison homologue insuffisante. Et ce défaut de réparation est accompagné d'une diminution de la résection de l'ADN. De plus, nous avons montré que la protection contre les dommages de l'ADN par TREX-2 dépend aussi de l'équilibre entre H2B et H2BUb1, contrôlé par le module de déubiquitination de SAGA.
Nos résultats démontrent une relation fonctionnelle entre TREX-2 et l'activité déubiquitinylase de SAGA chez l'Homme pour la réparation par recombinaison homologue des *DSBs*.
Mots clés: réparation de L'ADN, recombinaison homologue, TREX-2, GANP, ENY2, H2Bub1

Abstract in English

The maintenance of proper genetic information is essential to ensure correct cellular functions and to avoid genomic instability, which is a hallmark of cancer. In response to Double Strand Breaks (DSBs), cells initiate the DNA damage response (DDR), a signalling cascade that acts through two main sub-pathways: the error-prone Non-Homologous End Joining (NHEJ) and the error-free Homologous Recombination (HR). HR is promoted in S and G2 phases, when the availability of a sister chromatid allows the utilization of complementary genomic information to replace the lost DNA.
The Nuclear Pore Complex (NPC)-associated TREX-2 complex, is involved in transcription and mRNA export. In yeast TREX-2 has been implicated in genome stability maintenance through a mechanism that is dependent on gene-gating, connecting transcription and mRNA export at the NPC. This mechanism requires a connection between these two cellular processes that relies on a physical and functional interaction of TREX-2 with the co-activator complex SAGA.
The high evolutionary conservation of the TREX-2 complex between yeast and mammals strongly suggests that it might also protect human cells from genomic instability.
Here, we have investigated the role of TREX-2 in DSB repair in human cells. We find that loss of the scaffold subunit of TREX-2 (GANP) results in DNA repair deficiency by HR. This GANP-dependent HR defect is accompanied by decreased resection. Moreover, we showed that the mechanism through which TREX-2 protects human cells from DNA damage is dependent on an interplay with the SAGA deubiquitination module in maintaining a balance between histone H2B and H2Bub1.
Our results demonstrate a functional cross-talk between human TREX-2 and the SAGA deubiquitination activity that is important to ensure correct DSB repair during HR.
Keywords: DNA repair, Homologous Recombination, TREX-2, GANP, ENY2, H2Bub1