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Chapter 8

# Signalling DNA Damage

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. Types and sources of DNA damage

During our lifetime, the genome is constantly being exposed to different types of damage caused either by exogenous sources (radiations and/or genotoxic compound) but also as byproducts of endogenous processes (reactive oxigen species during respiration, stalled forks during replication, eroded telomeres, etc).

From a structural point of view, there are many types of DNA damage including single or double strand breaks, base modifications and losses or base-pair mismatches. The amount of lesions that we face is enormous with estimates suggesting that each of our $10^{13}$ cells has to deal with around 10.000 lesions per day [1]. While the majority of these events are properly resolved by specialized mechanisms, a deficient response to DNA damage, and particularly to DSB, harbors a serious threat to human health [2].

DSB can be formed [1] following an exposure to ionizing radiation (X- or $\gamma$-rays) or clastogenic drugs; [2] endogenously, during DNA replication, or [3], as a consequence of reactive oxygen species (ROS) generated during oxidative metabolism. In addition, programmed DSB are used as repair intermediates during V(D)J and Class-Switch recombination (CSR) in lymphocytes [3], or during meiotic recombination [4]. Because of this, immunodeficiency and/or sterility problems are frequently associated with DDR-related pathologies.

## 2. DNA repair of DSB. NHEJ and HR

Mammalian cells are equipped with two mechanisms that repair DSB and prevent dangerous chromosomal rearrangements; [1] *Non-homologous end joining (NHEJ)*, which links together the two ends of broken DNA by direct ligation, and [2] *Homologous Recombination (HR)*, which is only available S and G2 phases when a sister chromatid can be used as a template for the repair reaction (see Figure 1).

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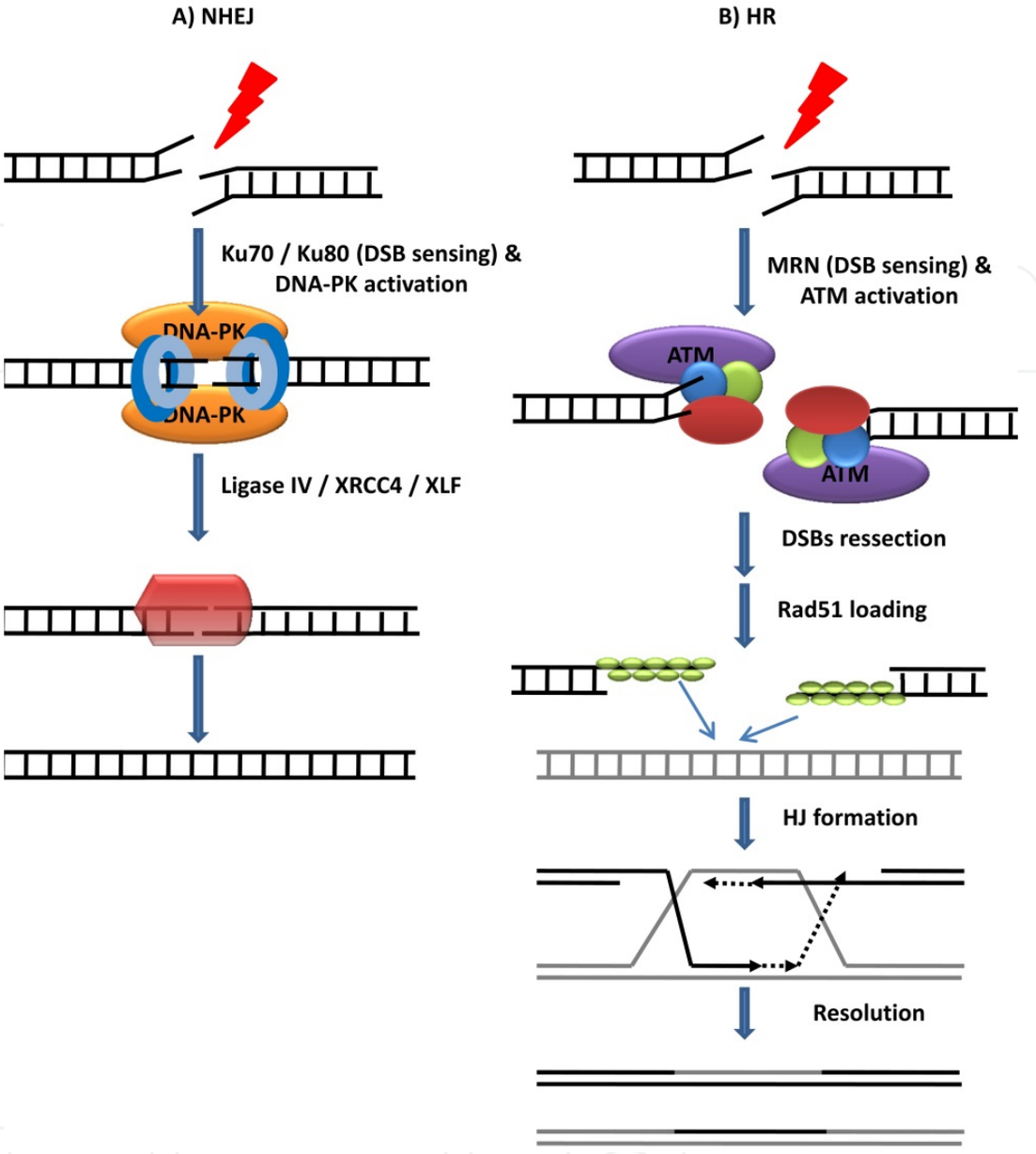



**Figure 1. DSBs repair mechanisms.** *A) NHEJ.* DSBs are sensed by the ring-shape heterodimer Ku70/Ku80 which then stabilizes the two DNA ends and recruits DNA-PK. Next, DNA-PK phosphorylates and activates the NHEJ effector complex (ligase IV/XRC44/XLF) that finally religates the broken DNA. *B) HR.* The ATM kinase is recruited to DSB via an interaction with the MRN (Mre11-Rad50-Nbs1) complex. Once at the break, ATM that becomes activated, phosphorylating multiple substrates. In a reaction that depends in multiple endo and exonuclease activities (including Mre11, Exo1 and CtIP) DSBs are resected forming ssDNA strands. These ssDNA regions attract Rad51 and other associated proteins. The Rad51-coated nucleoprotein filaments then invade the undamaged sister strands forming HJ structures. HR is completed by new DNA synthesis and still to be identified HJ "resolvase" enzymes.

## 2.1. Non Homologous End Joining (NHEJ)

NHEJ is simpler and faster than HR, and can take place in any cell cycle stage. However, NHEJ can process altered or improper ends is thus more error-prone that HR. Therefore, HR is the

preferred repair mechanisms during S/G2 phases. However, due to the preponderance of the G0/G1 stage, NHEJ is key to safeguard genomic integrity in mammalian organisms. Moreover, and probably due to the larger genomes and abundant repetitive sequences which limit HR proficiency, mammalian genomes are also frequently healed by NHEJ in S/G2 [5].

The mechanism by which NHEJ rejoins a DSB is fairly understood. First, DSB are recognized by a heterodimer formed by **Ku70** and **Ku80** proteins [6]. Ku proteins display a ring-like structure and are able to bind and stabilize the ends of DSB, acting as sensors, and subsequently attracting the transducer kinase, **DNA-PK** (DNA dependent protein Kinase). DNA-PK is a large Ser/Thr kinase (about 470 Kda), which belongs to the PIKK family (PI3-kinase like family of protein kinases). Other members of this family, ATM (ataxia talangectasia mutated) and ATR (ATM and RAD3-related), are key kinases in the DDR and will be explained in futher detail below. Once DNA-PK is located to the DSB it becomes activated and phosphorylates several substrates involved in the processing and ligation of DNA ends. Significantly, DNA-PK can also auto-phosphorylate in several residues, which seems to be essential for its repair capacity [7].

Finally, the main effectors of NHEJ form a multimeric complex (**DNA Ligase IV-XRCC4-XLF**), which is recruited to DSB and activated by DNA-PK dependent phosphorylation. This complex is responsible for the actual rejoining of the ends [8]. Ligase IV provides the catalytic activity for the rejoining, while XRCC4 stabilizes and stimulates its activity [9].

Ku70 and Ku 80 (sensors), DNA-PK (transducer) and DNA ligase IV and XRCC4 (effectors) are the core component of NHEJ. But often other accessory components are required for the conversion of altered broken ends. For instance, DSB produced by IR usually requires an end-processing step prior to relegation, such as the one regulated by **Artemis**, a 5'- 3' exonuclease, that acquires endonuclease activity when phosphorylated by DNA-PK [10]. This activity of Artemis is important to open closed hairpins in which the two strands of the DSB have linked together. Importantly, such hairpins are normally generated at the DSB generated by RAG nucleases during V(D)J recombination. Note that ATM can also induce the phosphorylation and activation of Artemis [11]. In fact, and in addition to be important for the repair of accidental DSB, NHEJ carries out several specialized functions in lymphocytes during V(D)J and CSR [12, 13]. Accordingly, patients with mutation in Artemis, ATM or DNA-PKcs, as well as in other NHEJ components, often suffer from immune deficiencies as well as from an increased predisposition to cancer [14].

Another context in which difficult-to repair DSB are formed relates to chromatin accessibility. For instance, it is estimated that around 10% of radiation induced DSB are repaired with slow kinetics, this population being enriched adjacent to compacted chromatin (heterochromatin). In this case, ATM and not DNAPK will play an important role in the NHEJ-mediated repair of these difficult-to-repair breaks. In this context, ATM activation is necessary to phosphorylate some substrates like H2AX or KAP1, which would relax chromatin compaction allowing the access of repair proteins to DSB at heterochromatin [11, 15]. Nevertheless, it should be noted that the main cell-type that suffers from ATM deficiency is Purkinje cells which are almost devoid of heterochromatin, so that ATM must play additional roles in euchromatin [16].

## 2.2. Homologous recombination

The other mechanism that cells use for DSB repair, and the most important for replicating cells, is Homologous Recombination. In contrast to NHEJ, during HR DSB need to be first extensively processed to allow for the search of the homologous undamaged template. First, ends undergo 5′-3′ resection producing two single stranded DNA regions. Rad51-coated ssDNA stretches invade the sister chromatid DNA duplex forming inter-strand structures. How these heteroduplexes are finally resolved is still not fully characterized.

Since HR repairs DSB using the sister chromatid as template, it is restricted to S and G2 phases. Restricting HR in G1 is in fact critical, since if HR takes place in G1 it could lead to loss of heterozigosity (LOH) or chromosomal translocations [17]. To avoid this problem the activity of several HR components is regulated by several S-G2 specific CDKs (cyclin dependent kinases) [18].

For teaching purposes, and trying to simplify the complex process that takes place during HR, we will artificially divide it in 4 independent stages. We acknowledge that many of the stepwise events described here are still to be fully validated, but just want to transmit a holistic view of how a DSB can be sensed, signaled and repaired by HR:

**a. Recruitment to the DSB: *Foci* forming factors**

The **Mre11-Rad50-Nbs1** (MRN) complex is one of the first in being recruited to DSB [19]. Next, through a direct interaction, MRN recruits **ATM** which, among other things, phophorylates H2AX (a variant of the canonical histone H2A) at serine 139, forming **γH2AX** [20]. Direct phospho-binding to γH2AX recruits the adaptor protein **MDC1** [21], which through interaction with ATM and Nbs1 forms a positive feedback loop that amplifies the γH2AX signal [22]. In addition, ATM-dependent phosphorylation of MDC1 is recognized by the E3 ubiquitin ligase **RNF8**, that ubiquitinates histones in the vicinity of the DSB [23, 24]. Another E3 ubiquitin ligase, **RNF168** (mutated in the human RIDDLE immunodeficiency syndrome), is also recruited and amplifies the ubquitylation signal, in both cases mediated by **UBC13** E2 ligase, eventually recruiting 53BP1 and BRCA1 to DSB-associated foci [25-27]. In the case of BRCA1, **RAP80** binds to ubiquinylated substrates at DSB through its UIM (Ubiquitin Interaction Motif) [23]. RAP80 then interacts with **Abraxas** [28], the deubiquitylating (DUB) enzyme **BRCC36** and **BRCA1** (breast cancer 1, early onset). BRCA1 is a well known human breast cancer susceptibility gene, responsible for the third part of familiar breast cancer [29]. BRCA1 is a large protein (over 200 kDa) that is constitutively bound to BARD1. Together, BRCA1/BARD1 present E3 ubiquitin ligase activity [30]. Although BRCA1 has been the subject of many studies and it is known to participate in HR, its exact role(s) and whether its ubiquitin ligase activity is critical for its activity is still not well understood. Whereas the road from DSB to BRCA1 is more understood, how 53BP1 is loaded to substrates modified by RNF8/RNF168 remains unknown.

Importantly, it should be noted that all of these events have been inferred from the formation or absence of *foci* at DSB and, to date, it is not clear to what extent capacity to recruit to foci represents an accurate measurement of the function of the protein [31].

**b. Processing of the end: Resection**

As mentioned before, the resection of the two 5' ends of the DSB is an essential step for HR. Three exonucleases (Mre11, Exo1 and CtIP) and one helicase (BLM) have been involved in resection [32-34]. Importantly, ATM dependent phosphorylation seems to stimulate CtIP-mediated resection, linking DNA damage signaling with the processing of DNA ends [35]. In addition to ATM, ssDNA is quickly coated by RPA (replication protein A) and triggers a parallel pathway resulting in the activation of the kinase ATR [18, 36-38], that will be described below. It remains to be seen to what extent ATM, ATR, and perhaps DNA-PK collaborate in the processing of DSB for HR. One important recent development is the finding that, against all expectations, 53BP1 might limit resection [39]. This would explain how the absence of 53BP1 rescues the cancer and ageing phenotypes of a BRCA1 mutant mouse model, which could be explained by an improved HR through enhanced resection [40].

**c. Sister chromatid invasion**

The initiation of recombination requires the loading of the **recombinase Rad51,** which replaces RPA coating on the ssDNA. Rad51, a protein with DNA-dependent ATPase activity, together with other associated proteins (DSS1, RAD52,RAD51B, RAD51C, RAD51D, XRCC2, XRCC3, RAD54, etc.) form a nucleoprotein filament that mediates strand invasion of the sister chromatid [41]. In mammals, Rad51 loading is mediated by **BRAC2** [42]. Once again, BRCA2 is another important breast cancer susceptibility gene suggesting a critical role of HR in the prevention of breast cancer [43]. In contrast to BRCA1, BRCA2 recruitment to DSB is not fully understood, one possible scenario being that BRCA1 could mediate the recruitment of **FancD2** and BRCA2 to DSB [44].

The nucleoprotein filament (ssDNA, RAD51 and other HR proteins) searches for an homologous region within the duplex DNA in the sister chromatid and displaces a DNA strand forming a D-loop structure and the 3' end of the invading strand. This invading end then is extended by un unknown DNA polymerase (*in vitro* data indicates that DNA pol eta may be implicated [45]). The "X" shaped structure formed as a consequence of this invading process is called a Holliday Junction (HJ) [46]. When the other 3' end of broken DNA enters the D-loop a double Holliday junction structure is formed. Noteworthy, whereas this model was proposed almost half a century ago, it was only recently that evidence for the double Holliday junction intermediates of repair was found [47]

**d. Resolution**

If HJ formation was remained elusive, the resolution of Holliday junctions is even a bigger mistery, many proteins having been proposed as candidates to be the HJ "resolvase". Such a protein should have helicase and nuclease activities, like BLM (Bloom syndrome protein), MUS81-EME1 complex or GEN1 [48-51]. All of them have been proposed to be the missing HJ-resolvase at some point. Moreover, recent evidence suggests that another enzyme, SLX4, could be such missing link [52, 53]. Whereas it seems clear that many proteins can deal with HJ-resembling structures *in* vitro, only clean genetic data will finally resolve this "resolution" problem. We should mention that although the HR classical model consists in the formation of these two Holliday Junctions, there are other alternative models that explain the HR process.

In addition to its role at DSB, HR also has a key role in restarting stalled replication forks (RF). SSBs, interstrand crosslinks or other lesions in the DNA promote stalling of the RF which, if persistent, could lead to breakage that has to be resolved by HR [54]. ATR, another PIKK kinase that is essential for an appropriate progression of RF, may be regulating HR in this case. In fact, ATR and its target kinase Chk1 phosphorylate a number of HR-related proteins such as BRCA1, BLM and Rad51 [55-57].

HR also participates in the maintenance of **telomere length**, by promoting inter-telomeric HR in the absence of telomerase. This pathway is known as alternative lengthening of telomeres (ALT) [58]. Finally, HR is the repair pathway of the recombination reaction that occurs between homologous chromosomes in **meiosis**, where DSB are intentionally generated in order to increase genetic variability.

Whereas we here provided a simplified view of DSB repair, it should be noted that other still poorly understood pathways of repair such as micro-homology directed NHEJ, which joins partially resected DSB, are emerging as important genome caretakers.

## 3. Signaling DSB

We here present a general view of how cells signal DSB, with a particular emphasis on phosphorylation, which is the most known mechanism to date.

### 3.1. Phosphorylation. The so-called DNA Damage Response (DDR)

The so-called DDR stands for a coordinated signaling response which starts at the DSB and which promotes DNA Repair while it limits the expansion of the damaged cell by apoptotic or cytostatic mechanisms. The canonical DDR begins with the activation of two PIKK, ATM and ATR, upon detection of DSB. The role of DNA-PK, another member of the PIKK family, is mainly to stimulate repair activities locally at the break but without triggering a cellular response. Thus, ATM and ATR are considered the main upstream kinases in the signaling of DNA damage. Whereas they are activated by different types of damage and have some specific substrates and functions, their "response" is frequently interconnected. The activation of any of these kinases starts a phosphorylation cascade leading to cell cycle arrest (*Checkpoints)*. Among others, the tumor suppressor p53 plays a central role in coordinating the apoptotic and checkpoints initiated by DNA damage.

In the last decade the knowledge about the DDR has substantially increased, and besides the classical activation of the PIKK and phosphorylation cascades, other types of signaling such as ubiquitylation or sumoylation have burst [59]. A general overview of this complex pathway will be explained below.

#### *3.1.1. Central players of the DDR: PIKK kinases: ATM, ATR and DNA-PK*

The DDR has a well defined hierarchy. Their components have been classified into sensors, transducers, mediators and effectors. ATM and ATR kinases are considered the main DDR transducers.

As mentioned, ATM, ATR and DNA-PK belong to the PIKK (phosphatidylinositol-3-kinase related kinases) family [60]. They are large proteins (more than 300 kDa) with similar structure: a variable number of repeat domains in the N-terminus (HEAT domains), a FAT domain, a catalytic domain homologue to that in PI-3-Kinase (PI3K), a PIKK regulatory domain (PRD) and a FATC domain at the very C-terminus [61]. The PRD domain has been shown to mediate kinase activation, at least for ATR; and HEAT, FAT and FATC domains may be involved in the specific interactions of these proteins with their substrates and modulators [62].

ATM, ATR and DNA-PK specifically phosphorylate serine and threonine residues frequently followed by a glutamine. Upon activation, they are able to phosphorylate hundreds of common substrates regulating many cellular functions [63]. Noteworthy, some of them are preferred by one specific kinase, which is likely due to mediators. For instance, whereas all three can phosphorylate H2AX, Chk1 is only phosphorylated by ATR and this is explained by the need of **Claspin** in mediating the ATR/Chk1 interaction.

Despite their similarities, these kinases play different roles in the DDR. In brief, DNA-PK is activated in response to DSB and promotes their repair by NHEJ. However, if DSB are located in heterochromatin or they are too numerous, ATM activates additional repair mechanism (e.g. HR) and starts checkpoints signaling. In addition, ATR is activated by ssDNA, generated when DSB persist and are ressected into ssDNA, or when a replication fork is collapsed, promoting a strong checkpoint signaling. Besides, whereas ATM and DNA-PK are active through all the cell cycle, ATR activity is confined only to S and G2 phases [64]. One possible scenario is that the activity of the three kinases follows a stepwise activation if a DSB persist. This model is explained below.

**a. DNA-PK. Stimulation of DNA Repair**

The first step in this model will be the immediate activation of DNA-PK at a DSB. Previously in this chapter, we described how DNA-PK is activated and promotes NHEJ. In summary, the dimer Ku70/Ku80 binds and stabilizes the DSB ends, it recruits DNA-PK that activates the NHEJ effectors, LigaseIV-XRCC4-XLF. Thus, DNA-PK will provide a fast repair mechanism for easy-to-repair breaks, without the activation of checkpoint activities. It must be emphasized again, that DNA-PK has a very limited role in checkpoint signaling. However in the absence of ATM, DNA-PK may stay longer at the DSB and contribute to checkpoint functions [65].

**b. ATM. DSB sensing and ATM Activation**

If the DSB persist, one plausible scenario is that ATM could phosphorylate and displace DNA-PK from the DSB, so that repair and checkpoint activities are implemented. ATM is the most studied and likely best known DDR kinase. One reason for this is that its absence is responsible for the human **Ataxia-Telangiectasia (A-T)** hereditary disorder [66]. A-T is a severe autosomal recessive disease characterized by early onset progressive cerebellar ataxia, oculocutaneous telangectasia, immunodeficiency and lymphoid tumours [67]. Immunodeficiency and predisposition to lymphoid tumours is explained by the role of ATM during T and B lymphocyte development, where DSB are generated as by-products of

immune rearrangements. Besides, cells of these patients present numerous breaks, what explains their increase predisposition also to other cancer types.

Whereas it seems clear that the main role of ATM is related to the DDR [68], the two symptoms that named the disease, ataxia and telangiectasia, are still not explained by the role of ATM in relation to DNA damage, suggesting that ATM may have other functions not related to DSB-signaling. Moreover, even though we have significant understanding of the effects of ATM activation, how this is accomplished is still a matter of debate.

As described above, ATM activation is triggered by the recruitment of the **MRN complex** (sensor) in response to DSB (Figure 2)**.** MRN complex recruits ATM and collaborates for its activation [69]. However, the precise mechanism by which ATM becomes activated is still a matter of controversy [62]. Some authors claimed that, in basal conditions, ATM is an inactive homodimer which undergoes dissociation and activation in response to DSB, due to its autophosphorylation [70]. Importantly, it has later been shown that ATM autophosphorylation is not necessary for its activation *in vivo* [71, 72].

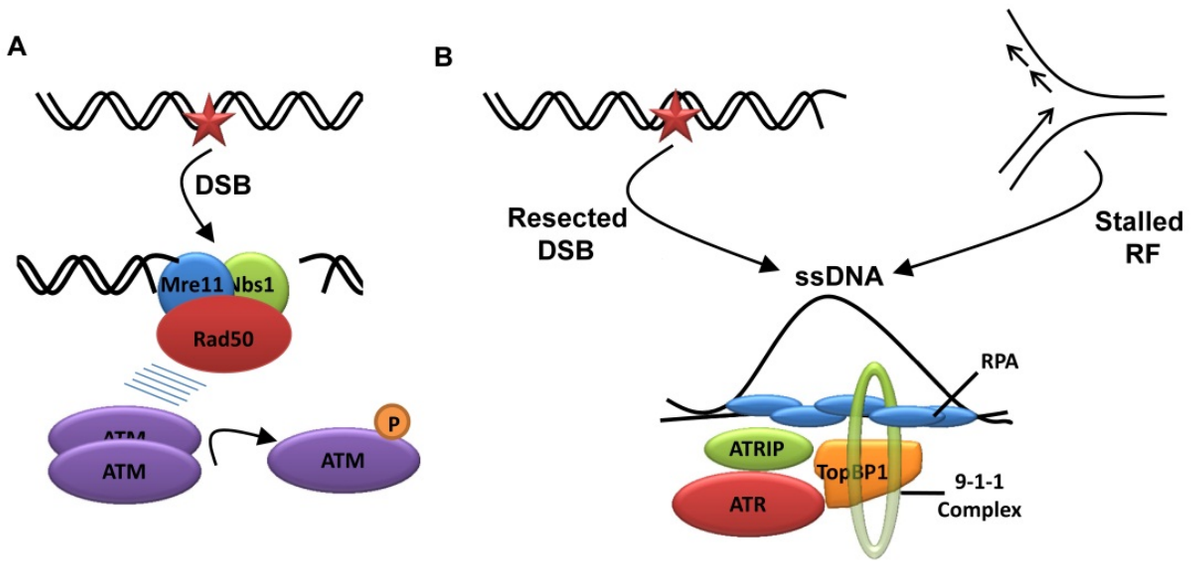



**Figure 2. Mechanisms of ATM and ATR activation.** *A) ATM activation*. DSBs (red asterisk) are sensed by the MRN (Mre11-Rad50-Nbs1) complex which attracts ATM and probably contributes to its activation. It is not well established how ATM is converted from its inactive to active status, one possibility being that it changes from an inactive dimer to an active monomeric form. *B) ATR activation*. The input for ATR activation is ssDNA, which can derive from resected DSB (left), or from stalled replication forks (right). RPA coated ssDNA loads the ATRIP-ATR complex and the 9-1-1 complex, which brings together ATR and its allosteric activator TopBP1.

Nbs1, part of the MRN complex, could contribute to ATM phosphorylation and activation [73], and several other proteins, such as the histone acetyl transferase Tip60 [74], have been reported to be involved in ATM activation. *In* vitro, ATM can be activated in the absence of MRN members. However, the MRN complex amplifies ATM activity and collaborates for an optimal ATM accumulation surrounding the DSB [75]. Noteworthy, defects in two members of the MRN complex cause A-T related syndromes: *Nijmegen breakage syndrome* (NBS1 mutants) and *A-T-like disorder* (MRE11 mutants) [76, 77].

ATM has been claimed to be activated by other stimuli rather than DNA damage, like chloroquine or osmotic shock [70]. However, whether those stimuli do not generate damage, particularly during replication, remains to be seen. There are some evidences of a cytoplasmic fraction of ATM that might be helpful to explain part of the symptoms of A-T disease not related to the DDR like the early neurodegeneration. Of note, ATM in neuronal cells has been found predominantly cytosolic [78].

**c. ATR. Activation by ssDNA**

The last (in fact, its activation occurs later than ATM) main PIKK kinase in the signaling of DSB is ATR. As noted before, despite their homology, ATR responds to different types of stresses than ATM does, and although these two kinases share several substrates, ATR regulates different processes. ATR has an essential role during replication, sensing alterations in fork progression and activating cellular checkpoints if necessary [79, 80].

In contrast to ATM, ATR is essential at the organism and the cellular level [81]. Consequently there is not any human disease lacking ATR. However, patients of a very rare ATR-related disease known as the Seckel syndrome are alive with very low amounts of ATR [82].

The input for ATR is not the DSB itself, but rather unusual large strands of ssDNA that can be generated in several circumstances [64]:

- RS (*replication stress*) is a not well defined concept that refers to a variety of alterations in the normal progression of the replication fork, caused by lesions encountered in the DNA (SSBs, crosslinks, base adducts), dNTPs deficiency or other problems at the RF. When RS is prolonged or, in the absence of ATR, RF collapse and DSB are generated. Notice that there is no such a thing as RS-free replication, and thus ATR is essential to complete replication even in the absence of exogenous DNA damage.
- *End resection of DSB*. Initially DSB lead to DNA-PK and ATM activation. However if DSB occurs in S or G2 phases and they persist enough, DSB are resected by ATM-stimulated exonucleases generating strands of ssDNA which then activate ATR.
- *Telomeres.* ssDNA can be also generated at uncapped telomeres that have lost its capping function, leading to ATR activation.
- *NER (nucleotide excision repair)*. The process of NER can generate patches of ssDNA as repair intermediates, which lead to the activation of ATR, perhaps even in G1 [83].

In contrast to ATM, the molecular mechanism of ATR activation by ssDNA is well established (Figure 2). **RPA-coated ssDNA** [84] recruits the **ATR-ATRIP** (ATR interacting protein) complex [85]. At the same time, **Rad17** and subsequently the **9-1-1** (Rad9-Rad1-Hus1] complex are also brought to the damage sites by RPA [86]. The 9-1-1 complex, with a PCNA-like clamp conformation, then brings the allosteric activator TopBP1 (topoisomerase-binding protein 1] into close proximity of ATR [87]. The interaction of ATR with **TopBP1** is then sufficient to unleash ATR activity [88]. Of note, activation of ATR by TopBP1 even in the absence of DNA breaks is sufficient to promote a robust cellular response including senescence, demonstrating the key role of the DDR in responding to DNA damage [89].

### *3.1.2. Mediators and DDR amplification. Foci forming factors*

Downstream of the PIKK, DDR signaling is amplified by several mechanisms, allowing the response to achieve its final cellular outcomes. On one hand, the DDR, like any other phosphorylation cascade, amplifies its signal in subsequent (enzymatic) step. This involves the participation of *mediators*, which are proteins acting downstream of the transducer kinases ATM and ATR. Several substrates, regulators, recruiters and others proteins acting as scaffold are considered mediators (e.g. Mdc1, 53BP1, MRN complex, Claspin, BRCA1), and, they modulate the activation of the effectors. Another mean of amplifying the signal, is an intense accumulation of many of their components surrounding the DNA lesions in spots so-called *IRIF (Ionizing Radiation Induced Foci)*.

Once ATM or DNA-PK are activated, the rapid phosphorylation of the histone H2AX (*γH2AX*) is key for subsequent events. The γH2AX mark is accumulated in such a large amount in the proximity of DSB that can be visualized in spots (IRIF) by standard immunofluorescence techniques. Many DDR proteins (MRN complex, ATM, 53BP1, BRCA1 and others) form IRIF that co-localize with γH2AX foci. Actually, γH2AX modulates the accumulation of repair and signaling proteins in chromatin regions distal to the DSB. However, this has nothing to do with the recruitment of the proteins to the DSB, and rather by altering chromatin conformation in the vicinity of the break [90]. But what are foci good for? One likely possibility is that the accumulation of DDR proteins surrounding DNA lesions is necessary to build a signaling threshold in conditions of limited damage [91].

Among the different factors, 53BP1 is probably one of the most studied examples of an IRIF forming protein. 53BP1 has a pan-nuclear location which, after exposure to genotoxic agents, is quickly repositioned to IRIF. 53BP1 localization into foci is dependent on several upstream events including H2AX phosphorylation [90], recruitment of MDC1, ubiquitinating activity of RNF8 and RNF168 [23, 25, 92, 93], and methylation of histones H3 and H4 [94, 95]. Regardless of their importance for our understanding of the DDR, the absence of foci does not seem to be essential for a proficient DDR. Thus, mouse models lacking H2AX or MDC1, that abolishes IRIF formation, as well as 53BP1 null mice, are viable, but they exhibit phenotypes related to DDR deficiency like genome instability, cancer predisposition or immunodeficiency [22, 96, 97]. In summary, while not essential, IRIF seem to modulate the amplitude of the signaling of DSB which might be important in conditions of low numbers of DSB.

In contrast to ATM, ATR does not undergo any post-translational modifications which would modify its activity, such as autophosphorylation. Nevertheless, a number of mediators of ATR signaling are indeed regulated through phosphorylation. For instance, the ATR activator TopBP1 is phosphorylated by ATM at resected DSB [98]; and probably also by ATR in response to RS. In addition, Claspin phosphorylation is also required for ATR-dependent phosphorylation of Chk1 in response to DSB [99].

In summary, ATM and ATR signaling are amplified and driven by these and many other mediators. The endpoint of the signaling cascade arrives with the activation of the effectors, which would finally be responsible for the cellular responses.

### *3.1.3. Effectors and cellular outcomes of the DDR*

In addition to promote the repair of DNA lesions, the DDR can orchestrate multiple cellular responses orientated to safeguard genome integrity or, in some cases, to avoid transmission of harmful alterations by activating apoptosis or senescence. In this context, one of the main effectors of the DDR is the transcription factor p53 which provides a late sustained response to DNA damage. p53 up-regulation contributes to the activation of checkpoints, and, if the damage persists, may activate senescence, apoptosis or cell differentiation programmes.

Regardless of this p53-centric view, it is clear that many p53-independent pathways are also stimulated by the DDR. For instance, a single proteomic study identified more than 700 ATM/ATR phosphorylation substrates, pointing out the wide variety of effectors and pathways that are regulated by the DDR [63].

Given that there are multiple reviews elsewhere that focus on these cellular responses, we here only briefly describe the main cellular responses promoted by the DDR: cell cycle checkpoints, senescence, apoptosis and differentiation.

#### *3.1.3.1. Transient cell cycle delay: Checkpoints*

The transitions through the different stages of the cell cycle are tightly regulated by the activity of cyclin-dependent kinases (CDKs). CDKs are activated by cyclins and inhibited by CDK inhibitors (CKIs) or inhibitory tyrosine phosphorylations [100]. In brief, a simplified scheme depicts that four CDKs are involved in the regulation of cell cycle; CDK2, CDK4 and CDK6 during interphase, and CDK1 being considered the mitotic CDK. An activated DDR can limit the activity of CDKs and thus prevent cell cycle progression into the next stage (G1/S and the G2/M). In addition, the DDR can also slow down replication (intra-S checkpoint), but this is not a full stop and cells with damage in S-phase progress into G2 stop at the G2/M checkpoint [101]. The way that the DDR gets to the CDKs is through effectors that limit CDK activity which we now briefly summarize.

First, the main PIKK targets that regulate checkpoints are the so-called checkpoint kinases Chk1 and Chk2, direct substrates of ATR and ATM respectively [102, 103]. Checkpoint kinases act by regulating CDK inhibitory effectors such as Cdc25a, Wee1 or p53. *Cdc25*a is a phosphatase that controls CDK1 and CDK2 activities. In response to DNA damage, ATR-activated Chk1 phosphorylates Cdc25a inducing its degradation [104, 105], and thereby, inhibition of CDK activity [106]. An opposing force for Cdc25a is *Wee1*, the kinase that counteracts Cdc25a activity. Finally, a major mediator of cellular responses to DNA damage is p53. *p53* is rapidly stabilized upon DNA damage by a number of inter-dependent PTMs. ATM, ATR, Chk1 and Chk2 are all able to phosphorylate p53 contributing to its stabilization [107, 108]. Besides its apoptotic targets, p53 has a number of transcriptional targets that contribute to checkpoint function, perhaps the most notorious being the CKI $p21^{cip1}$ which is an important regulator of the G1/S checkpoint.

Specifically, the ***G1/S checkpoint*** avoids the replication of damaged DNA (see Figure 3 for a scheme of checkpoint responses). The transition from G1 to S is directed by an increase in CDK2 activity induced by cyclin E [109]. As such, the G1/S checkpoint is directed to limit

this burst in CDK activity. An early and late component checkpoint can be separated. First, the G1/S checkpoint is initiated by degradation of Cdc25a, leading to the accumulation of inhibitory phosphorylations on CDK2 [110, 111]. This is an early response that does not require the synthesis of new proteins. If the damage persists, p53-dependent transcriptional upregulation of $p21^{cip1}$ would implement a stronger G1/S arrest [112].ATM and Chk2, but not ATR/Chk1 are the main DDR mediators of the G1/S transition.

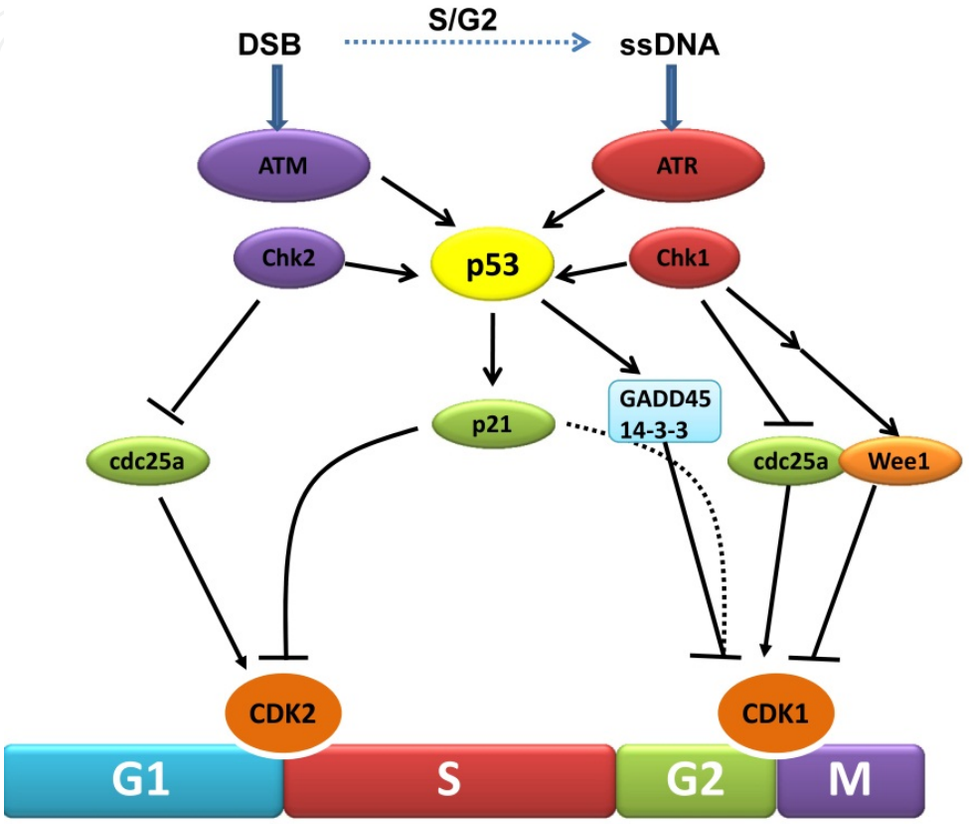



**Figure 3. Chekpoint regulation by the DDR**. ATM and ATR orchestrate a transitory delay of the cell cycle in response to DSBs and ssDNA, respectively. Wherease direct phosphorylation of of cdc25a and wee1 allow a rapid establishment of the G1/S and G2/M checkpoints, p53-dependent regulation contributes to checkpoint maintenance at later timepoints. During S and G2 phases DSBs can be resected leading to the generation of ssDNA, which also activates ATR-signaling.

If damage arises in S phase, the ***intra-S checkpoint*** is also mainly regulated by the phosphatase Cdc25a, but now under de control of ATR and Chk1 [102]. In addition, DDR-promoted replication inhibition is in part mediated by Cdt1, which participates in the loading of MCM helicases that facilitate DNA unwinding ahead of the RF [113]. How the DDR promotes Cdt1 degradation and thus replication control is yet uncharacterized.

The last key control point is the ***G2/M checkpoint***, that "checks" genome integrity before proceeding into chromosome segregation. Here, the transition from G2 into M is governed by an increase cyclinB-CDK1 activity. Once again, this checkpoint is also established by Cdc25a phosphorylation, in this case mediated by the ATR-dependent Chk1 kinase. In addition, the kinase Wee1 also contributes to the G2/M checkpoint. In a normal G2/M

transition Plk1 (Polo kinase 1) phosphorylates Wee1 inducing its degradation and entry into mitosis. Upon damage ATM and ATR are able to phosphorylate and inhibit Plk1, contributing to an increase of Wee1 and therefore reduced CDK1 activity [100]. In addition to this early checkpoint, p53 also regulates the maintenance or sustained G2/M arrest. In this case, rather than mediated by p21, which has little CDK1 inhibitory capacity, other p53 targets such as GADD45 and 14-3-3 act as effectors [114].

*3.1.3.2. Permanent cell cycle arrest: Senescence*

By definition, checkpoints are a transitory state from which cells can escape if repair is accomplished and the signal is turned off. In contrast, cellular senescence is a persistent and irreversible cell cycle arrest. It was firstly observed in cell culture in 1965 [115]. Depending on the load of DNA damage or the cell type, a persistent activation of the DDR might direct cells into senescence [89].

Besides the presence of DSB, other cellular stresses can also promote senescence. These include telomere shortening [116], oxidative stress [117] and oncogenes [118-121]. Nevertheless, all of these stimuli can activate the DDR, so that DNA damage might be the ultimate cause of the senescent response. First, short or unprotected telomeres resemble and are actually sensed by cell as DSB [122, 123]. Second, reactive oxygen species generated by oxidative stress are an obvious source of DNA damage. Finally, several oncogenes have been shown to induce RS so that oncogene-induced senescence (OIS) would be linked to the DDR [124-126]. Importantly, and as it will be discussed below, OIS has been suggested as an early anti-cancer barrier in vivo, providing a link between the DDR and cancer development [127]. However, it must be pointed out that alternative, DDR-independent pathways of promoting OIS and which operate through the Ink4a/ARF locus have been described [128].

*3.1.3.3. Apoptosis*

Apoptosis, or programmed cell death, is other phenomenon activated by the DDR in order to eliminate cells with intolerable amounts of DNA damage. As such, apoptosis has an important role in eliminating damaged cells during aging [129] or in the acute responses genotoxic cancer therapies. In addition, apoptosis is a physiological process essential for normal development.

As in the case of senescence, apoptosis is also chiefly governed by p53. DDR-induced p53 promotes the expression of several pro-apoptotic factors, such as Puma, Noxa and Bax [130-132]. This pro-apoptotic p53 program leads to mitochondrial membrane permeabilzation [133], allowing the exit of cytochrome c to the cytosol, forming the apoptosome, which finally activates effector caspases [134].

Why some cells undergo apoptosis and other senescence in response to DNA damage is still a matter of study [135]. Although most cells are capable of both phenomenons, cell type is indeed determinant to undergo one or another. For instance, whereas DNA damage in fibroblasts and epithelial cells specially promotes senescence, low amounts of DNA breaks in lymphocytes are sufficient to trigger apoptosis. Of note, post-mitotic cells like neurons, have a limited capacity to become senescent or undergo apoptosis which might be due to their particular cell cycle status.

*3.1.3.4. Other cellular pathways stimulated by the DDR*

Given the large amount of PIKK phospho-targets and p53 transcriptional targets it is not surprising that the DDR exerts its function in many cellular processes by means that remain poorly understood. For instance, high doses of DNA damage can induce the *differentiation* of melanocyte stem cells (SC), resulting in their depletion and thereby promoting aging phenotypes on the skin [136]. Similar results linking the DDR and differentiation have been found in neurons [137]. Related to this, p53 has been shown to regulate the *polarity* of mammary cancer stem cell divisions [138]. p53 loss promotes symmetric and promiscuous cancer stem cell divisions, which contribute to the expansion of premalignant pools. It is therefore tempting to speculate that one of the means by which DNA damage leads to stem cell exhaustion is by promoting p53-dependent asymmetric cell divisions in SC.

Finally, another process that might be modulated by DNA damage is *autophagy*, which functions to promote cell survival through the degradation of damaged organelles and molecules. Interestingly, one of the key regulators of autophagy is mTOR (mammalian target of Rapamycin), another member of the PIKK family. Whereas the mechanism remains to be solved, recent studies suggest a connection between the DDR and mTOR that could be the responsible for DNA damage induced autophagy [139, 140]. To what extent these additional effects of the DDR impact on the physiological consequences of a deficient DDR, remains to be seen.

### *3.1.4. Physiological consequences of the DDR*

DDR deficiencies cause important physiological consequences. As commented on this chapter, DSB are physiologically generated during B and T lymphocyte maturation and during meiotic recombination, and many DDR-related proteins are required for normal functioning of these processes. In addition, the DDR has an essential role in facing the stochastic DNA damage that our cells acquire through our lifetimes. In this context, it is not surprising that deficiencies in DDR components cause human diseases associated to immune deficiencies, sterility, premature aging and cancer predisposition. Alterations during embryonic development are also frequent, suggesting that the high division rates occurring at this stage might be prone to accumulate DNA damage, particularly RS.

DDR-mutant mouse models recapitulate many of the phenotypes found in DDR-associated human syndromes. Some of the most relevant DDR-related diseases are shown in **table 1**. Most of them are of recessive nature, with some exceptions such as variants of Li-Fraumeni, which can be caused by dominant mutations in just one allele of p53.

*3.1.4.1. The DDR and cancer: Protector and target*

The relationship between the DDR and cancer is of particular interest from multiple points of view. First, the DDR is critical **to prevent the accumulation of spontaneous pro-cancerous mutations** and overall genomic instability. This is why most DDR-related human diseases are prone to cancer development. In addition, the DDR is particularly relevant in preventing chromosome rearrangements during lymphoid maturation which makes lymphomas one of the most frequent cancer in Genomic Instability Syndromes. Note that in some cases mutations

| Disease | Mutated gen | Clinical feature |
|---|---|---|
| *Ataxia Telangiectasia* | ATM | Cerebellar ataxia, telangiectasia, inmunodeficiency, lymphoid tumours. |
| *Nijmegen breakage syndrome* | Nbs1 | Growth retardation, microcephaly, immunodeficiency, lymphoid tumours. |
| *A-T like disorder* | Mre11 | Cerebellar ataxia, mild predisposition to tumours. |
| *Seckel Syndrome* | ATR | Progeria, microcephaly and other developmental defects. |
| *Li-Fraumeni* | p53 | Early development of cancer (breast cancer, sarcomas, brain tumours, leukemias, etc.) |
| *Riddle syndrome* | RNF168 | Inmunodeficiency, dysmorphic features, mental retardation. |
| *Werner syndrome* | RECQL2 (WRN) | Progeria and age-associated disorders |
| *Fanconi Anemia* | 13 different FA genes | Bone marrow failure, predisposition to leukemias and solid tumours. |

**Table 1.** Several human genetic syndromes related to DDR components.

in only one allele convey a dramatic increase in cancer predisposition. This is the case for BRCA1 and BRCA2 mutations carriers, whose life-time breast cancer risk raise up to 80%.

Second, **the DDR is activated by oncogenes** in early stages of tumorigenesis [124, 141]. These studies proposed a model where the activation of certain oncogenes could generate RS which, by activating the DDR, would limit cancer development by promoting cell senescence [142, 143]. In fact, convincing evidence exists to show that certain oncogenes indeed are able to generate DNA damage trough promoting abnormal replication [124, 125]. Note that this type of damage, known as RS, is sensed and signaled mainly by ATR rather than ATM [83], raising the relevance of ATR-signaling in the oncogene-activated DDR model of cancer progression.

Finally, many of the current **anti-cancer therapies** (including radiotherapy) operate by generating high loads of DNA damage that activate the DDR towards apoptosis. In this regard, there is increasing interest in the development of new anti-cancer strategies that take advantage of our knowledge of the DDR to specifically target tumor cells. The idea behind these new strategies is to exploit synthetic lethal interactions that will only occur in cancer cells. For instance, one of the most promising approaches in this regard is the use of *PARP inhibitors* for the treatment of BRCA1/2 deficient breast cancers. These inhibitors block a ssDNA repair pathway which is mostly dispensable for normal cells, but essential for cells deficient in HR, such as BRCA1/2 mutant cells [144]. Other examples of synthetic lethality could be the use of Chk1 inhibitors and ATR inhibitors, to treat p53 deficient tumors [145-148].

*3.1.4.2. The role of the DDR in ageing*

Ageing is intuitively associated with the natural degeneration of our tissues, which would derive from the accumulation of some "toxic" factor. Studies mostly performed in the last decade have identified DNA damage as this deleterious factor that is associated to the onset of ageing [149, 150]. For instance, aged tissues or stem cells show evidences of an activated DDR [151, 152]. Moreover, most DDR-related genetic diseases suffer from premature ageing, which is likely due to a faster accumulation of intolerable amounts of DNA damage.

The most accepted theory is that DNA damage, when accumulated in SC, activates a DDR that limits their regenerative capacity and thus promotes ageing [151, 153]. To what extent DNA damage is the natural cause of actual ageing in humans, and which types of DNA damage (RS, eroded telomeres, ROS…) are most important in this process, remains to be understood. In what regards to RS, the faster nature of embryonic cell divisions might make this stage particularly susceptible to this type of damage. In fact, recent studies in ATR hypomorphic mice revealed that an intra-uterine exposure to RS can accelerate the later onset ageing [146, 154]. Whereas the exact mechanism of this intrauterine programming of ageing is not fully understood, it raises the question about to what extent our adult well-being can be already conditioned by the stresses to which we were exposed *in utero*.

## 3.2. Other posttranslational modifications in the DDR

There is little doubt that PIKK-mediated phosphorylation is a major controller of the DDR. Nevertheless, to end this chapter we would want to describe the role that non-phosphorylation based signaling of DNA damage might play in genome protection. In the light of recent discoveries, DNA damage signaling through other PTMs such as acetylation, methylation, ubiquitination and, sumoylation might also play crucial roles for appropriate DNA damage signaling.

### *3.2.1. Ubiquitination*

Ubiquitination is a highly regulated process that promotes covalent modification of specific proteins substrates with the 76-amino acid protein *ubiquitin*, catalyzed in three sequential steps by *E1* (*ubiquitin-activating enzyme*), *E2* (*ubiquitin-conjugating enzyme*) and E3 (*ubiquitin ligase*) enzymatic activities [155]. Classically, ubiquitination (UQ) was described as a mechanism to target proteins for proteasome mediated degradation. However, at present, other functions of UQ such as during protein localization or in mediating protein-protein interactions are known. In fact, phosphorylation signaling (not only by the DDR) is often coupled to substrate ubiquitination, which increases the potential network of protein-protein interaction [156, 157].

UQ presents seven lysines in its surface: K6, K11, K27, K29, K33, K48 and K63. Whereas poly-ubiquitination linked to K48 residues is usually related to proteasomal degradation [158], K63-linked polyubiquitin chains are more related to other regulatory functions [159]. In addition to the conjugating lysine, ubiquitin chains display an ample structural diversity, that has its counterpart in many different *ubiquitin interactif motifs (UIM)* in other proteins [160]. The role of UQ is counteracted by *deubiquitianting enzymes (DUB)*, which are responsible for the elimination of UQ conjugates.

Several ubiquitination events have been assoctaied to DSB signaling. As previously discussed, PIKK activation is followed by ubiquitination of various substrates. Again, ATM-dependent phosphorylation of H2AX and MDC1 are sufficient to recruit the E3 ubiquitin ligase *RNF8*. Together with another E3 ligase known as RNF168, and with *Ubc13* as the E2 ligase, RNF8 promotes the accumulation of ubiquitinylated substrates in the vicinity of the DSB. Of note,

*RNF168* loading to foci occurs trough direct recognition of RNF8-deposited UQ chains through its UIM domains [25, 26]. These poly-ubiquitinations are mainly K63-linked and contribute to attract specific UIM-containing proteins and/or to modulate chromatin architecture via histone ubiquitinylation [159]. Recent works have shown that HERC2 (Hect Domain and RLD2) is an important regulator of RNF8 and RNF168-dependent ubiquitinitalion which mediates through promoting the binding to the E2 ligase Ubc13 [161].

This coordinated cascade of UQ modifications generated at DSB is necessary for the formation of BRCA1 and 53BP1 foci. However, once again, the absence of RNF8 does not lead to major BRCA1 phenotypes, which challenges the relevance of IRIF as predictors of functionality. It should be noted also that there is no evidence of direct binding of 53BP1 to ubiquitin chains, and how UQ mediates 53BP1 foci remains a mistery. In addition, 53BP1 can bind methylated histones which may provide an independent way of loading to DSB [94, 95, 162].

In contrast to 53BP1, the relationship of BRCA1 foci with UQ is well characterized. *RAP80*, a protein containing several UIMs in tandem, binds to ubiquitinated substrates (perhaps histones) [23], and brings Abraxas [28] and BRCA1. Interestingly, RAP80 also interacts with *BRCC36*, a DUB, which activity is also required for proper DSB repair and signaling [163, 164]. Thus, ordered ubiquitinylation and deubuquitinylation may be needed during the signaling of DNA damage.

The UQ pathway does not end with 53BP1 or BRCA1. *BRCA1* itself is an E3 ubiquitin ligase activity resident in the N-terminal RING domain of this large protein. In fact, this is the only enzymatic activity found in BRCA1. While BRCA1 has E3 activity by itself, this activity increases in several orders of magnitude when bound to its constitutive partner BARD1 [30]. To date, the only well characterized BRCA1 substrates are BRCA1 itself and CtIP. CtIP ubiquitination by BRCA1 [165] has been proposed to promote the resection of DSB, leading to HR and a regulation of the G2/M checkpoint through ATR-activity. Hence, in this system UQ would be upstream of the phosphorylation DDR.

To complicate things further, BRCA1 auto-ubiquitination is linked to K6, which is mediated by the E2 enzyme Ubch5c [166]. Ubiquitinated BRCA1 is found at DSB and also in several endogenous spots during S phase. Interestingly, even though 20% of human BRCA1 mutations predisposing to breast and ovarian cancer are found in the RING domain [30], recent data emerging from mouse models suggest that the E3 ligase activity of BRCA1 might be unrelated to genome maintenance and its tumor suppression role [167, 168]. However, other studies [169, 170] indicate the importance of the RING domain in BRCA1 tumor suppressor function. Certainly, further work needs to be done to solve these divergent observations.

Finally, regardless of DSB there is solid evidence that UQ-mediated signaling pathways also contribute to other genome protective pathways. For instance, UQ plays a key role in the *Fanconi Anemia* pathway. Fanconi Anemia (FA) is a recessive disease characterized by developmental abnormalities, bone marrow failure and cancer predisposition [171]. The disease is caused by mutations in at least 13 genes which mutations compromise the repair of interstrand DNA cross-links. A key event on the activation of the FA pathway is the monoubiquitination of FancD2 and FancI proteins, with is thought to be essential for its

localization at chromatin [44, 172, 173]. Again, there is evidence to suggest that FANCD2 ubiquitinylation is downstream of ATR signaling, providing a further example of the constant interaction between the different signaling pathways that are activated by DNA damage [55].

### *3.2.2. SUMOylation*

SUMO stands for Small Ubiquitin-like modifier due to its similarities with UQ. In fact, SUMOylation is a similar process to ubiquitinylation involving E1, E2 and E3 enzymes. Importantly, very few SUMO E3 ligases exist in the mammalian proteome, which limits the search for potential ligases. In addition, SUMO E3 ligases are mostly dispensable and E2 ligases can complete the SUMOylation reaction largely by themselves. In contrast to UQ, three SUMO variants (SUMO1, SUMO2 and SUMO3 exist [174].

A number of SUMO roles with genome maintenance pathways have been discovered. In what regards to the DDR, two recent studies identified a SUMO-related signaling cascade that also coordinates the foci formation of BRCA1 and 53BP1 in response to DSB [175, 176]. At present, *PIAS1* (*Protein Inhibitor of Activated STAT-1*) and *PIAS4*, are the E3 SUMO ligase enzymes found to participate in the DDR, using *SAE1* (*SUMO activating enzyme subunit 1)* as E1 and *UBC9* (Ubiquitin Conjugating Enzyme 9) as E2 enzymes. All these proteins, as well as SUMO1 and SUMO2/3 conjugates are rapidly recruited to DSB, forming IRIF. Whereas the mechanism is still not totally clear, it seems that PIAS1 promotes SUMO2/3 modification of BRCA1, and PIAS4 is mainly involved in SUMO1 modifications of both 53BP1 and BRCA1. Together, these SUMOylations seem to be necessary for RNF8 and RNF168 function, providing another instance of inter-PTM signaling at DSB [175, 176]. A summary of key SUMO and UQ modifications in the DSB response is illustrated in **table 2**.

| E3 Ligase | E2 Ligase | Main substrates | Function |
|---|---|---|---|
| | | **Ubiquitinations** | |
| *FancL* | Unknown | FancD2 | DNA Cross-link repair |
| *BRCA1 / BARD1* | Ubch5c | BRCA1 | Unknown, |
| | | CtIP | DSB resection |
| *RNF8* | Ubc13 | H2A, H2AX | DSB signaling |
| *RNF168* | Ubc13 | H2A, H2AX | DSB signaling |
| | | **SUMOylations** | |
| *PIAS1* | Ubc9 | BRCA1 | DSB signaling |
| *PIAS4* | Ubc9 | BRCA1, 53BP1 | DSB signaling |

**Table 2.** Relevant ubiquitinations and SUMOylations in genome maintenance.

Regardless of the DDR, other genome maintenance pathways are also controlled by SUMO. One example is the role of a SUMO ligase called Mms21, which is an essential component of a cohesin and condensin related complex formed by Smc5 and Smc6 [177]. Whereas the role of this complex is far from being understood, it seems it might be important to prevent the accumulation of hemicatenates at stalled replication forks [178].

### *3.2.3. Interplay of PTMs in the DDR*

Other post-translational modifications, such as methylation and acetylation, are also involved in the regulatory network of the DDR. A paradigm of multiple and interconnected PTM is found in the **regulation of p53** levels, that involves a coordinated network of phosphorylation, acetylation, methylation and ubiquitination [179].

In the absence of DNA damage, p53 levels are kept low due to its *ubiquitination* by the E3 UQ ligase MDM2 and rapid degradation by the proteasome [180]. In fact, one of the ways by which DNA damage and other stress signals promote p53 stabilization is by PTMs which alters the MDM2/p53 interaction. p53 also suffers DDR-dependent *phosphorylations* [107] are thought to stabilize the protein and allow its association with the CBP/p300 acetyltransferase complex, promoting p53 *acetylation* and further stabilization [181]. Besides, p53 activity is limited by Set8/Pr-Set7 mediated methylations [182], which are also upon DNA damage [183].

Another relevant example of the interplay between different PTMs in the DDR is found in **histones**. Indeed, post-translational modifications of histones regulate many other processes related to chromatin compaction and structure, such as replication and transcription. The best known histone modification upon DNA damage is the already described *phosphorylation* of H2AX, that it is mediated by ATM, ATR or DNA-PK [20]. We also discussed how RNF8 and RNF168 can *ubiquitinate* histones in the proximity of DSB [26, 92]. Finally, *methylations* of histones H3 and H4 could modulates the recruitment of 53BP1 [94, 95, 162]; and several *acetylations* of histones have been described to play roles in genome maintenance which are still not completely understood [184-186]. How histone modifications enhance DSB repair is a very active area of research and discussion. One possibility is that histone modifications could increase chromatin accessibility, which would therefore facilitate the repair and signaling of the breaks [187].

## 4. Future perspectives

We have here provided a general overview of how cells signal the presence of DNA damage, and how a proper signaling is necessary to maintain a healthy genome. Still, whereas the amount of PTMs that coordinate the cellular response to DNA damage is already intimidating, it is likely that we are only seeing the tip of the iceberg. Many other targets and even PTM (ADP-rybosylation, Neddylation, N-terminal glycosylation, etc…) will probably be involved in mounting a proper DDR. Without a doubt, the fast development of massive proteomic technologies will soon provide a breathtaking picture of how cells detect, signal and repair DNA breaks; by promoting a myriad of PTMs in almost every molecule involved in the DDR.

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