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Abstract

The view of DNA packaging into chromatin as a mere obstacle to DNA repair is evolving. In this review, we focus on histone variants and heterochromatin proteins as chromatin components involved in distinct levels of chromatin organization to integrate them as real players in the DNA damage response (DDR). Based on recent data, we highlight how some of these chromatin components play active roles in the DDR and contribute to the fine-tuning of damage signaling, DNA and chromatin repair. To take into account this integrated view, we revisit the existing Access-Repair-Restore model and propose a new working model involving Priming chromatin for Repair and Restoration as a concerted process. We discuss how this impacts on both genomic and epigenomic stability and plasticity.

Introduction

Genome integrity is constantly challenged both by environmental agents and by metabolic products that can induce DNA damage (Ciccia and Elledge, 2010). The cellular response that follows, termed the DNA damage response (DDR), is a coordinated series of events that allows DNA damage detection, signaling (including cell cycle checkpoint activation) and repair (Jackson and Bartek, 2009; Ciccia and Elledge, 2010; Giglia-Mari et al., 2011). In contrast with programmed events such as DNA replication in S phase, the DDR has to be elicited at any place and any time, where and when DNA lesions occur. Importantly, the DDR should not be considered just at the DNA level but in the context of chromatin in eukaryotic cell nuclei, where DNA is wrapped around histone proteins (Kornberg, 1977) and associates with non histone components that promote higher-order fiber folding (Probst et al., 2009; Li and Reinberg, 2011). Studies over the past decades have emphasized the critical importance of chromatin components, whose nature and spatial organization are sources of information that contribute to cellular function and identity (Probst et al., 2009; Li and Reinberg, 2011). Thus, we need to consider how the integrity of this information is challenged due to the reorganization of chromatin upon DNA damage (Groth et al., 2007b). Early observations showing a transient increase in nuclease sensitivity in chromatin regions undergoing repair after UV irradiation on human fibroblasts (Smerdon and Lieberman, 1978) provided the foundations for a model referred to as "Access-Repair-Restore" (ARR) (Smerdon, 1991). This model describes basic aspects of chromatin reorganization following DNA damage: damaged chromatin first becomes more accessible to enable DNA repair, followed by restoration of chromatin organization (Green and Almouzni, 2002; Groth et al., 2007b). More recent work provided further insights into the mechanisms that ensure

accessibility to damaged chromatin by exploiting the concerted action of chromatin remodeling factors and histone modifying enzymes (reviewed in Greenberg, 2011; Luijsterburg and van Attikum, 2011; Lukas et al., 2011; Polo and Jackson, 2011; Deem et al., 2012). Upon completion of DNA repair, restoration of chromatin organization relies on mechanisms involving histone chaperones and remodeling factors (De Koning et al., 2007; Ransom et al., 2010). Notably, in this overall scheme, chromatin is envisaged as a barrier to repair that needs to be lifted and then put back. However, recent evidence shows that chromatin components can also actively promote DNA damage signaling and repair. Thus, it is of interest to revisit the ARR model to further integrate this new dimension of the response. Herein, we review the DDR from the nucleosome level up to higher-order chromatin structures by focusing on histone variants and heterochromatin proteins, and we highlight how these components can behave as active players in the DDR pathway. Although distinct repair mechanisms are at work depending on the type of damage and the cell cycle stage, for simplicity we focus mainly on the response to DNA double-strand breaks (DSBs) to illustrate the interplay between the DDR and chromatin dynamics.

The role of histone variants in shaping damaged chromatin

The nucleosome - the fundamental unit of chromatin - comprises a core particle, in which DNA is wrapped around a histone octamer composed of a $(H3\text{-}H4)_2$ tetramer flanked by two H2A-H2B dimers (Dechassa et al., 2011). Connecting adjacent particles, linker DNA associates with linker histones (Happel and Doenecke, 2009). This repeated motif shows variations in its composition due to the combinations of PTMs (post-translational modifications) that can be added to each histone and to the existence of distinct histone variants (Probst et al, 2009). Histone variants are

paralogs presenting variations in their amino-acid sequences that range from a few residues to entire domains. Importantly, histone variants differ in their expression throughout the cell cycle and in their localization within chromatin (Talbert and Henikoff, 2010; Boyarchuk et al., 2011; Szenker et al., 2011). During all their cellular life, histone variants are escorted by specific chaperones (De Koning et al., 2007). In concert with ATP-dependent remodeling factors (Clapier and Cairns, 2009), these chaperones can mobilize histones in and out of chromatin. The discovery of the phosphorylation of the H2A variant, H2A.X, as one of the earliest events in response to DNA damage (Rogakou et al., 1998) provided the first hint of the importance of histone variants in the DDR. In this section, we review our current knowledge of histone variant dynamics upon genotoxic stress considering the variants with reported roles in the DDR, namely H2A, H3 and H1 variants. We also highlight how these dynamics contribute both to a fine-tuning of the DDR and to the maintenance of chromatin integrity.

Functional importance of H2A variant dynamics in the DDR

To date, the three H2A variants with documented roles in the DDR are H2A.X, H2A.Z and MacroH2A (Table 1).

H2A.X

As mentioned above, H2A.X is rapidly phosphorylated at DSB sites (Rogakou et al., 1998), and its phosphorylated form - known as γH2A.X - is widely used as a marker for DDR activation. Signaling of DSBs starts with γH2A.X recruiting MDC1 (Mediator of DNA damage chekpoint 1), which is critical for further recruitment of other checkpoint mediators such as 53BP1 (p53 binding protein 1) and BRCA1 (Breast cancer 1, early onset). Bidirectional spreading of γH2A.X away from DNA

breaks helps to amplify the checkpoint signal (Yuan et al., 2010) and thereby contributes to delineate a chromatin region where the DDR is confined (Figure 1). Although how this confinement is achieved and whether there are defined boudaries is unclear, recent reports using high-resolution profiling of γH2A.X highlight that γH2A.X spreading is a discontinous process influenced by gene transcription and cohesin binding (Iacovoni et al., 2010; Caron et al., 2012). Notably, besides phosphorylation of H2A.X, its acetylation and ubiquitylation also contribute to the recruitment of DDR proteins to damage sites (reviewed in Yuan et al., 2010). These DNA damage-dependent modifications do not only affect H2A.X. Indeed, the histone chaperone FACT (facilitates chromatin transcription), initially identified for its ability to mobilize H2A-H2B during transcription (Belotserkovskaya et al., 2003) and which also mediates H2A.X exchange, gets poly(ADP-ribosyl)ated following genotoxic stress (Du et al., 2006; Heo et al., 2008). As a consequence, H2A.X dynamics can be altered as follows: *i)* Post-translationally modified H2A.X is more prone to dissociate from nucleosomes than the unmodified form (Ikura et al., 2007; Heo et al., 2008) and *ii)* H2A.X/H2A exchange is inhibited by FACT poly(ADP-ribosyl)ation, a modification that disrupts FACT interaction with nucleosomes (Du et al., 2006; Heo et al., 2008). Additional factors can also contribute to H2A.X dynamics, including the chromatin remodeler and modifier Tip60 complex (Tat-interactive protein 60), which in Drosophila acetylates phospho-H2Av (an H2A.X/H2A.Z ortholog) and promotes its replacement by unmodified H2Av (Kusch et al., 2004). Tip60 may act similarly in human cells by acetylating H2A.X to increase its mobility (Ikura et al., 2007). In contrast, the turnover of budding yeast phospho-H2A (which resembles γH2A.X) can be counteracted by the action of remodeling factors such as the remodeling complex INO80 (Inositol requiring 80) (Papamichos-Chronakis et al., 2006). While these

studies delineate a complex network of factors that control H2A.X dynamics in response to DNA damage to fine-tune the checkpoint signal (Figure 1), an important issue that remains to be addressed is to which extent γH2A.X replacement, as opposed to γH2A.X dephosphorylation, contributes to checkpoint termination. In fact, this question also applies to checkpoint activation: is it only due to the modification of histones already in place or should we also consider modification of H2A.X prior to its incorporation into chromatin? Novel tools to follow histone dynamics that distinguish new and old histones *in vivo,* such as the epitope-tag switch in yeast and the SNAP-tag technology in mammalian cells (Jansen et al., 2007; Verzijlbergen et al., 2010; Ray-Gallet et al., 2011), provide experimental means to monitor H2A.X dynamics upon DNA damage. In addition, examining how H2A.X dynamics can be regulated by H2A.X modifications in cooperation with histone chaperones and remodelers is another issue that warrants further investigation. The genetic system recently established in Drosophila that allows the replacement of the whole histone gene cluster by mutated histone transgenes, thus preventing or mimicking a histone modification (Günesdogan et al., 2010), opens up exciting avenues to address these issues. In conclusion, the modulation of H2A.X modifications and its dynamics offer a tunable switch to turn DDR signaling on and off, and to coordinate DDR events at the chromatin level.

H2A.Z

Besides H2A.X, H2A.Z contribution to the maintenance of genome integrity has emerged mainly based on studies in budding yeast. Indeed, knocking-out H2A.Z or factors promoting its dynamics leads to an increased DNA damage sensitivity (Shen et al., 2000; Michael S Kobor et al., 2004; Mizuguchi et al., 2004; Luk et al., 2007;

Kalocsay et al., 2009) and double mutant yeasts lacking both H2A.Z and the checkpoint kinase Mec1 (Mitosis entry checkpoint protein 1) exhibit synthetic sensitivity to DNA damage (Bandyopadhyay et al., 2010). Among factors involved in H2A.Z dynamics, the remodeling complex SWR1 (Sick with Rat8 ts; Mizuguchi et al., 2004) promotes the replacement of nucleosomal phospho-H2A (γH2A.X) by H2A.Z (Papamichos-Chronakis et al., 2006; Figure 1). Furthermore, H2A.Z deposition by SWR1 close to a persistent DSB promotes DSB resection (Kalocsay et al., 2009). In an antagonistic manner, the remodeling factor INO80 stimulates H2A.Z replacement by H2A (Papamichos-Chronakis et al., 2011; Figure 1). Thus, INO80 and SWR1, which both bind to DSBs and can assist DSB repair (reviewed in Morrison and Shen, 2009) regulate DNA damage signaling in a dynamic way. In addition, acetylation of H2A.Z may also control its DDR function given the fact that mislocalized unacetylated H2A.Z is a source of genome instability (Bandyopadhyay et al., 2010; Papamichos-Chronakis et al., 2011). Since remodeling factors involved in H2A.Z dynamics are evolutionarily conserved and have been involved in the DDR in human cells (reviewed in Morrison and Shen, 2009; Luijsterburg and van Attikum, 2011), whether similar mechanisms also operate in mammals should be investigated. Also, it may be worth exploring the significance of H2A.Z presence in pericentromeric domains in mammalian cells (reviewed in Boyarchuk et al., 2011) as a potential remnant of previous repair events in regions presenting a repetitive DNA structure.

MacroH2A

This highly divergent H2A variant comprises two paralogs - macroH2A.1 (with two spliced forms) and macroH2A.2 - characterized by the presence of a large carboxy-terminal macrodomain that is known to function as an ADP-ribose binding module

(Karras et al., 2005). Notably, covalent modification of proteins with ADP-ribose polymers takes place at sites of DNA breaks catalyzed by enzymes of the PARP (Poly(ADP-ribose)polymerase) family (Hakmé et al., 2008), which promotes the recruitment of several proteins with affinity for ADP-ribose (Lukas et al., 2011; Polo and Jackson, 2011). Interestingly, only the macroH2A.1.1 isoform is able to bind ADP-ribose *in vitro,* and its isolated macrodomain - but not the full length protein - accumulates at laser-induced DNA damage sites *in vivo* (Timinszky et al., 2009; Table 1). This accumulation is impaired by treatment of cells with PARP inhibitors (Timinszky et al., 2009) and involves the histone chaperone APLF (Aprataxin and PNKP-like Factor) (Mehrotra et al., 2011). Although the exact contribution of macroH2A to the DDR is unclear, the possibility that macroH2A could trigger local chromatin compaction at damage sites (Timinszky et al., 2009) deserves further investigation. Interestingly, in addition to distinct poly(ADP-ribose) binding properties, macroH2A variants also display both tissue and cell-type specific expression. MacroH2A.1.1 is expressed mostly in non proliferating cells (Pehrson et al., 1997; Costanzi and Pehrson, 2001), which may contribute to a distinct cellular response in terms of chromatin rearrangements in response to DNA damage. In future studies, it will be important to clarify the impact of macroH2A variants on chromatin compaction at DNA damage sites, how it interconnects with ADP-ribose metabolism and how it may impact on the DDR.

Involvement of H3 variants and their specific chaperones in the DDR

Three main categories of H3 variants can be distinguished: the replicative variants - H3.1 and H3.2 in mammals - that ensure a large provision of histones in S phase, the replacement variant H3.3, constitutively expressed throughout the cell cycle and in

quiescent cells, and the specific centromeric variant CenH3 (also known as CENPA, centromere protein A, in mammals) (for review see Talbert and Henikoff, 2010; Szenker et al., 2011; Table 1).

Replicative variant H3.1

Compared to H2A variants, the dynamics of H3 variants in the DDR has only received attention recently. This can be due to their lower mobility as measured by FRAP (Fluorescence Recovery After Photobleaching) experiments in cells (Kimura and Cook, 2001) along with the fact that specific antibodies for H3 variants were not available. However, transient expression of epitope-tagged H3.1 variants in human cells revealed new histone deposition at both UVC- and laser-induced damage sites in a CAF-1 (Chromatin Assembly Factor-1)-dependent manner (Polo et al., 2006; Figure 1). CAF-1, initially identified as a factor stimulating histone deposition during replication (Stillman, 1986), was also shown to restore chromatin organization on repaired DNA *in vitro* (Gaillard et al., 1996), and later defined as a dedicated chaperone for H3.1 deposition (Tagami et al., 2004; Ray-Gallet et al., 2011). Thus, H3.1 deposition is not restricted to replication but also takes place at sites of DNA repair synthesis *in vivo*. Most importantly, new histone deposition at DNA damage sites implies that there is not a simple recycling of pre-existing histones, and this potentially helps to replace old histones. An open issue is how to control the amount of new histones to be loaded. This may relate to the extent and type of damage on the DNA, and perhaps also to the damage of histone proteins themselves, which could trigger their elimination (Bader and Grune, 2006). Importantly, new soluble histones differ from pre-existing nucleosomal histones in terms of their post-translational modifications (Loyola et al., 2006) and as a consequence their incorporation into

chromatin will dilute parental marking. This alteration of local chromatin marks may subsequently affect the expression of genes in the damaged chromatin region. This possibility is supported by recent findings in the context of DNA replication in chicken cells deficient for the specialized polymerase REV1 (Reversionless 1) involved in the bypass of roadblocks such as G4-DNA sequences. In these cells, the uncoupling of DNA replication and histone recycling at the time of replication leads to a local increase of new histone incorporation and thereby a loss of parental marks, which alters the transcriptional status of the loci (Sarkies et al., 2010; 2011). In light of these findings, a tight control of histone recycling along with new histone deposition is likely critical. In this context, the histone chaperone ASF1 (Anti-Silencing Function 1) involved in both recycling parental histones and providing new histones at the replication fork (Groth et al., 2007a; Jasencakova et al., 2010) will be interesting to examine for a similar function at repair sites.

As described above, CAF-1-mediated H3.1 deposition is important to restore nucleosomal organization at damage sites. Interestingly, in response to DNA damage, the depletion of CAF-1 mid-subunit does not perturb activation of DNA damage signaling (Polo et al., 2006). However, there is an interesting connection between chromatin restoration and termination of DNA damage signaling, as shown in budding yeast where CAF-1 ortholog contributes to the recovery from checkpoint arrest (Kim and Haber, 2009). Therefore, new H3.1 histone incorporation coupled to DNA repair participates in the restoration of nucleosomal organization after DNA damage and possibly modulates checkpoint termination (Table 1, Figure 1).

Centromeric variant CenH3

Zeitlin et al. recently detected the centromeric H3 variant CenH3 at DNA damage sites induced by laser micro-irradiation or endonuclease cleavage in mammalian cells (Zeitlin et al., 2009; Table 1, Figure 1). Considering that mislocalized CenH3 may lead to aberrant centromere formation, the functional relevance of its accumulation at damage sites remains unclear. Intriguingly, the recently identified CenH3 histone chaperone HJURP (Holliday junction recognition protein; Dunleavy et al., 2009; Foltz et al., 2009; Shuaib et al., 2010) had also been connected to the DDR. Indeed, it was initially characterized as a protein that binds to Holliday Junctions (recombination intermediates) *in vitro* and whose expression increases after DNA damage in human cells in a manner that depends on the DSB sensor kinase ATM (Ataxia telangiectasia mutated; Kato et al., 2007). Although it is not known if HJURP contributes to CenH3 deposition at damage sites, HJURP and CenH3 expression levels correlate with cell sensitivity to radiation *in vitro* and *in vivo*, high levels of HJURP being predictive for increased sensitivity to radiotherapy in breast cancer patients (Hu et al., 2010). Together, these data emphasize the need to further characterize HJURP and CenH3 properties to determine whether distinct or similar features as those required for their centromeric function could be at work in the DDR.

Besides CenH3 and H3.1, the dynamics of other H3 variants in response to DNA damage remains an open question (Table 1, Figure 1). Interestingly, a potential role for H3.3 dynamics in the DDR can be inferred from work in fission yeast. The ortholog of the HIRA (Histone regulator A) complex, a critical H3.3 chaperone, is required for protection against genotoxic agents, as shown by mutating the Hip1, Slm9 and Hip3 subunits (Anderson et al., 2009). The possible link between H3.3 and genome stability is further supported by the recent identification in high grade

pediatric brain tumors and in pancreatic tumors of mutations in H3.3 itself as well as in DAXX-ATRX (Death domain associated protein-α-thalassemia/mental retardation syndrome X-linked), another H3.3-specific chaperone (Jiao et al., 2011; Schwartzentruber et al., 2012; St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project et al., 2012). Given that stretches of naked DNA can get exposed by nucleosome rearrangements in damaged chromatin without being necessarily associated with DNA synthesis, H3.1 deposition mechanism may not suffice. It will be particularly interesting to investigate whether the nucleosome gap-filling function recently proposed for human H3.3 as a salvage pathway for chromatin integrity (Ray-Gallet et al., 2011) applies to sites of DNA damage. Stretches of naked DNA exposed by nucleosome rearrangements in damaged chromatin would then be prone to H3.3 deposition. How the distinct H3.3-specific chaperones including the HIRA and DAXX complexes identified in mammalian cells work in this context should be investigated. Moreover, given the interconnected dynamics reported for H3.1, H3.3 and CenH3 in human cells (Dunleavy et al., 2011; Ray-Gallet et al., 2011), it will be important to examine possible cross-talks/compensatory mechanisms between H3 variant dynamics at damage sites.

Role of linker histone variants in the DDR

Multiple variants of linker histone H1 have been identified (Happel and Doenecke, 2009) but their specific functions in the DDR are poorly characterized.

Out of the six H1 variants expressed in chicken cells for example, only H1R has been involved in protecting cells from DNA damage and could possibly participate in the DSB response, as indicated by its contribution to sister chromatin exchange rates (Hashimoto et al., 2007). In mouse cells, a reduced amount of H1, obtained by

deleting the genes encoding H1.2, H1.3 and H1.4 variants, leads to hyper-activation of the DNA damage checkpoint and cells show an enhanced resistance to several DNA damaging agents (Murga et al., 2007). In budding yeast, a null mutation in the H1-related gene *HHO1* also enhances DNA damage resistance while its overexpression enhances damage sensitivity by inhibiting recombinational repair (Downs et al., 2003). Whether there are species specificities for H1 contribution to the DDR, or distinct roles for the distinct variants remains to be elucidated, as well as their actual mode of action, whether it exploits modulation of nucleosome fiber folding or other means.

Together, this section highlights the dynamics of histone variants and the importance of their specific chaperones and associated remodelers essentially at all steps in the DDR. More specifically, they contribute to early DNA damage signaling, to DNA repair, to fine-tune and amplify checkpoint signals, to restore chromatin organization after repair and finally to turn off checkpoint signals. Beyond the crosstalks described above within one class of histone variant (e.g. H2AX/H2AZ and H3.1/H3.3/CenH3), possible inter-connections between variants of different classes should also be considered in future studies to fully comprehend nucleosomal dynamics in the DDR. Indeed, the different types of nucleosome particles (and sub-particles) generated by the combination of distinct variants, whose availability varies throughout the cell cycle, may be critical to control the efficiency of DNA damage signaling and repair. Finally, while histone variants are core components of the nucleosome, they also mark distinct chomatin regions and with their PTMs provide binding sites to anchor other chromatin proteins involved in higher-order structure (Probst et al., 2009). This

leads us to the next question, which is how the dynamics of these other chromatin proteins relates to the dynamics of histones and contributes to the DDR.

Contribution of higher-order chromatin organization and non-histone chromatin proteins to the DDR

The issue of the dynamics of non-histone chromatin proteins during DNA damage signaling and repair is just emerging. In the following section, we summarize first our current understanding of how higher-order chromatin organization can impact the DDR with key examples of heterochromatin regions. Second, we explore how, beyond their function in heterochromatin organization, particular heterochromatin proteins could also have general roles in promoting the DDR.

Heterochromatin versus euchromatin: distinct spatio-temporal dynamics of the DDR

According to Emil Heitz's definition, heterochromatin, as opposed to euchromatin, remains densely stained throughout the cell cycle (Heitz, 1928). To denote regions that are similarly treated on the two homologous chromosomes and do not change status during development, Spencer Brown called them constitutive as opposed to facultative heterochromatin (Brown, 1966). Today, by analysing combinations of histone modifications genome-wide, a further sub-classification of chromatin regions could be derived (de Wit et al., 2007; Filion et al., 2010). At this stage, we will consider the simple historical distinction between euchromatin, generally gene-rich and transcriptionally active, and heterochromatin, that is gene poor, rich in repetitive sequences and essentially transcriptionally silent (Maison and Almouzni, 2004). In

light of these particular features, the key questions concerning the DDR in heterochromatin are (i) whether a higher level of compaction in these regions could impose particular constraints on the DDR, (ii) whether heterochromatin as a barrier limits access to all DDR factors, (iii) whether the repetitive nature of heterochromatin DNA with its high recombination potential requires a tighter regulation of the DDR.

In budding yeast, the higher-order chromatin packaging involving the non-histone proteins Sirs (Silent information regulators) has proven useful to start approaching these issues (Gasser and Cockell, 2001). Sir proteins inhibit recombinational repair at the strand invasion step, yet chromatin remodelers such as SWI/SNF (SWItch/Sucrose NonFermentable) can overcome this inhibition (Sinha et al., 2009). In mammals, histone acetyltransferases participate in activating the DDR (Murr et al., 2005), a requirement bypassed by inhibiting histone deacetylases (HDACs), which induces global chromatin relaxation (Kim et al., 2007; Murga et al., 2007). Both local and global relaxation of chromatin can be detected upon DNA damage induction (Kruhlak et al., 2006; Ziv et al., 2006; Dellaire et al., 2009). Furthermore, the energy-dependent relaxation of chromatin observed upon local DNA damage induction (Kruhlak et al., 2006) suggests that active remodeling is required to promote DNA repair within chromatin. Collectively, these observations stress that chromatin packaging as a structural constraint requires chromatin remodelers and histone modifying enzymes to allow access to DNA damage (reviewed in Luijsterburg and van Attikum, 2011), as initially envisaged in the ARR model. The choice of mouse cells, in which pericentric heterochromatin forms chromocenters easily detected by their DAPI-dense staining and highly enriched in classical heterochromatin marks such as HP1 (Heterochromatin Protein 1; Probst et al., 2009), proved very useful to study the DDR

in heterochromatin. When mouse cells are arrested in G0/G1, γH2A.X foci disappear with slower kinetics in regions surrounding chromocenters, suggesting a slower DSB repair in heterochromatin when compared to euchromatic regions (Goodarzi et al., 2008). Given the high enrichment of HP1 proteins in these domains, whether their presence could impede DNA repair at these loci was explored (Ayoub et al., 2008; Goodarzi et al., 2008). The HP1 protein family in mammals comprises three related paralogs HP1α, β and γ that exhibit roles ranging from gene silencing and heterochromatin organization to transcriptional activation (Fanti and Pimpinelli, 2008; Kwon and Workman, 2011). All HP1 proteins present a chromodomain in their N-terminal region that recognizes histone H3 tri-methylated on Lysine 9 (H3K9me3), a histone modification that is highly enriched in heterochromatin (Maison and Almouzni, 2004). In addition, in HP1 C-terminal portion, a domain closely related to the chromodomain, named chromoshadow interacts with several partners including HP1 proteins themselves thus allowing homo- and hetero-dimerization (Nozawa et al., 2010; Rosnoblet et al., 2011). In response to DNA damage in mammalian cells, Ayoub at al. reported a release of HP1 β from regions enriched in H3K9me3 (Ayoub et al., 2008). This release involves phosphorylation of HP1β chromodomain by CK2 (casein kinase 2) and promotes efficient H2A.X phosphorylation (Ayoub et al., 2008). Furthermore, the simultaneous knockdown of all HP1 paralogs or of the enzymes that establish the H3K9me3 mark alleviate the repair defect observed in heterochromatin (Goodarzi et al., 2008), thus suggesting that HP1 proteins act negatively on the DDR. Similarly, recent findings in *Drosophila* showed that knockdown of the HP1α ortholog or of H3K9 methylases facilitate accumulation of late DNA repair proteins in heterochromatin (Chiolo et al., 2011). Interestingly, DNA repair in the chromocenters of cells arrested in G0/G1 also requires the ATM kinase to trigger the

release of the HP1 binding factor KAP-1 (KRAB-associated protein 1; Goodarzi et al., 2008). KAP-1 phosphorylation by ATM disrupts KAP-1 interaction with the nucleosome-remodeling factor CHD3 (Chromodomain helicase DNA-binding protein 3), leading to the displacement of CHD3 from heterochromatic DSBs (Goodarzi et al., 2011). How CHD3 displacement facilitates repair is still unclear, although the proposed model (Goodarzi et al., 2011) suggests that this involves chromatin relaxation. Together, these findings highlight the importance of specific mechanisms to counteract the steric hindrance associated with higher-order folding in heterochromatin and allow efficient repair.

Besides its negative impact on repair, heterochromatin can also affect the DDR at the signaling level. In budding yeast, γH2A.X cannot spread into a 'heterochromatic sequence' inserted within a euchromatic region (Kim et al., 2007). Similarly, in mammalian cells, γH2A.X spreading in response to ionizing radiation is blocked when running into heterochromatin regions (Jakob et al., 2011). In support of an inhibitory effect of heterochromatin, high-resolution imaging of chromocenters showed that DDR markers such as γH2A.X are not detected within heterochromatin domains but rather at their periphery in response to global or localized damage (Cowell et al., 2007; Kim et al., 2007; Goodarzi et al., 2008; Jakob et al., 2011). However, examination of ionizing radiation- and laser-induced damage at early time points unveiled that the generation of DNA breaks and the initial steps of DNA damage signaling and repair (i.e. H2AX phosphorylation and the recruitment of MDC1, RPA (Replication protein A) and XRCC1 (X-ray repair cross-complementing 1)) take place efficiently within the core of heterochromatin domains in both *Drosophila* and mammalian cells (Baldeyron et al., 2011; Chiolo et al., 2011; Jakob et al., 2011). This is followed by the relocalization of damaged DNA to the periphery of

the heterochromatin domains, where later steps of DNA damage signaling and repair (i.e. γH2A.X spreading and RAD51 recruitment) likely proceed in a more permissive environment (Chiolo et al., 2011; Jakob et al., 2011). Thus, heterochromatin compartments do not necessarily significantly obstruct the initial steps of the DDR. Indeed, large molecules can diffuse in heterochromatin domains (Bancaud et al., 2009). Interestingly, although ATM is important for the repair kinetics of DNA lesions in heterochromatin, the exclusion/relocalization of damaged DNA proceeds normally in the absence of functional ATM (Goodarzi et al., 2008; Jakob et al., 2011). ATM independent mechanisms must thus be considered to explain the relocation of damaged DNA. This possibly relates to the expansion of heterochromatin domains observed in response to DNA damage, both in *Drosophila* (Chiolo et al., 2011) and mouse cells (Baldeyron et al., 2011). This expansion may allow protrusion of damaged DNA into the surrounding euchromatin to finalize later steps of the DDR in a more accessible chromatin environment. Whether this kind of relocalization of DNA damage allows DSB clustering should fuel the debate concerning the existence of repair centers (reviewed in Misteli and Soutoglou, 2009).

We summarize these findings in a working model for DNA repair within pericentric heterochromatin domains in higher eukaryotes (Figure 2), which should stimulate investigations for other types of heterochromatin including facultative heterochromatin. Strikingly, during replication, a similar repositioning is observed for replicating DNA at the periphery of pericentric heterochromatin domains (Quivy et al., 2004). This remarkable parallel suggests that similar mechanisms have evolved to displace damaged or replicating DNA from inner heterochromatin compartments to confine activities at the periphery of the domain. It is conceivable that such mechanisms, by restricting the processing of DNA ends at the periphery, avoid

ectopic recombination between repetitive sequences within heterochromatin (Quivy et al., 2004; Peng and Karpen, 2008; Chiolo et al., 2011). This could in turn prevent deleterious chromosomal rearrangements and genomic instability.

The paradox of heterochromatin proteins in the DDR: besides a repressive role, an active contribution?

Several recent reports showed that heterochromatic/repressive factors including HP1 (Luijsterburg et al., 2009; Baldeyron et al., 2011), PcG (Polycomb group) proteins (Hong et al., 2008; O'Hagan et al., 2008; Chou et al., 2010; Ismail et al., 2010; Ginjala et al., 2011; O'Hagan et al., 2011; Šustáčková et al., 2012), KAP-1 (Ziv et al., 2006; Baldeyron et al., 2011) and HDAC1/2 (Miller et al., 2010; Polo et al., 2010) get recruited to DNA lesions. Here, we present these data and discuss how the in and out dynamics of these proteins may be exploited positively for the DDR.

HP1 proteins

HP1 proteins represent a good example to illustrate the above-described paradox. In apparent contrast with the findings described in the previous section that highlighted the inhibitory role of HP1 during the DDR, recent reports demonstrated that all HP1 paralogs get recruited to various types of DNA damage (Luijsterburg et al., 2009; Zarebski et al., 2009; Baldeyron et al., 2011). Furthermore, an efficient response to genotoxic agents in worms requires HP1 proteins (Luijsterburg et al., 2009) and HP1α contributes to homologous recombination in mammalian cells (Baldeyron et al, 2011). This suggests that these heterochromatin proteins could have a dual role in the DDR. Interestingly, HP1 accumulation at damage sites is rapid and transient and does not require H3K9me3 and/or other features usually associated with its

enrichment/retention at pericentric heterochromatin (Luijsterburg et al., 2009; Baldeyron et al., 2011). This is consistent with the fact that HP1 recruitment to damage sites does not require its chromodomain and rather depends on the chromoshadow domain (Luijsterburg et al., 2009). Importantly, *C. elegans* knocked-out for the HP1 ortholog hpl-2 (HP1-like 2) show a high sensitivity to ionizing radiation (Luijsterburg et al., 2009), revealing an active role of HP1 in DSB repair. This role is likely conserved based on further analyses in mammalian cells. HP1 targeting to damaged sites in mammals involves the interaction of HP1 chromoshadow domain with the largest subunit of the histone chaperone CAF-1 (Baldeyron et al., 2011; Figure 3). Notably, human cells depleted from HP1α show decreased survival after ionizing radiation, defects in the recruitment of DDR factors downstream of MDC1 - including 53BP1 and BRCA1 - as well as of repair factors such as the recombinase RAD51. The reduction in efficiency of homology-directed repair may result from impaired DNA-end resection (Baldeyron et al., 2011). This should be further explored to better understand how HP1 proteins contribute to the DDR. For example, it will be interesting to examine the potential involvement of candidate factors among the recently identified partners of HP1 including chromatin remodelers (i.e. BRG1 (Brahma-related gene 1), SMARCA2 *(*SWI/SNF related, matrix associated, actin dependent regulator of chromatin A2), CHD4) and proteins involved in sister chromatid cohesion (i.e. NIPBL (Nipped-B-like protein) and SGOL1 (Shugoshin-like 1)) (Nozawa et al., 2010; Rosnoblet et al., 2011). How HP1 modifications may affect this process is another exciting possibility, especially in light of the recently reported sumoylation of HP1 (Maison et al., 2011), and the importance of the SUMO (small ubiquitin modifier) pathway in the DDR in mammalian cells (Galanty et al., 2009; Morris et al., 2009). Importantly, HP1 family

members - α, β and γ in mammals - do show distinct properties (Kwon and Workman, 2011) and thus should also be considered for their individual roles in the maintenance of genome integrity.

To explain the dual roles of HP1 proteins in response to DNA damage, different models have been proposed. HP1 dynamics in response to damage could either be drastically different within heterochromatin and euchromatin (release vs. recruitment) or it may simply involve an extra step in heterochromatin where release of HP1 precedes its accumulation at damage sites (Ayoub et al., 2009; Dinant and Luijsterburg, 2009; Cann and Dellaire, 2011). However the comparable timing of HP1 recruitment to DNA damage sites both in heterochromatin and euchromatin within the same nucleus (Baldeyron et al., 2011) prompts us to reconsider these views. We thus propose a model where the overall dynamics of HP1, involving both release and recruitment, could promote DNA damage signaling and repair (Figure 3). In this scenario, a DNA damage-dependent release of HP1 proteins from H3K9me3 could contribute to increase the pool of HP1 available to be recruited elsewhere to damage sites. HP1 binding may be exploited in the DDR to promote local chromatin configurations that help stabilize loose ends and/or keep sister chromatids in proximity after the induction of DSBs. Moreover, a release of HP1 from H3K9me3 sites would expose this heterochromatin mark to allow other proteins to bind, such as Tip60, whose binding to H3K9me3 is required for activating its acetyltransferase activity (Sun et al., 2009). In addition, the recently reported loss of HP1 proteins in BRCA1 deficient cells (Zhu et al., 2011) invites to further examine the crosstalk between BRCA1, heterochromatin proteins and the DDR. In conclusion, instead of either recruitment or release of HP1, it may be important to consider the overall dynamics of these proteins.

Other heterochromatin proteins

Similar to HP1, other heterochromatin factors are recruited to DNA damage sites, where they actively contribute to the DDR (Figure 3). The HP1 binding factor KAP-1 also accumulates at damage sites, and the accumulation/retention of HP1 and KAP-1 are interdependent (Ziv et al., 2006; Baldeyron et al., 2011; Figure 3). This suggests that HP1 and KAP-1 roles in the DDR may be linked, although the mechanism underlying their mutual recruitment awaits further investigation. Likewise, core and accessory proteins of PRC1 (Polycomb repressive complex 1) and PRC2 also accumulate at damage sites, where they promote trimethylation of H3K27 and ubiquitylation of H2A and γH2A.X (Hong et al., 2008; O'Hagan et al., 2008; Chou et al., 2010; Ismail et al., 2010; Ginjala et al., 2011; O'Hagan et al., 2011; Šustáčková et al., 2012). The increased sensitivity to ionizing radiation consistently observed upon PcG protein knockdown further suggests an importance in DSB repair pathways. The PRC2 accessory factor PHF1 (PHD finger protein 1) for example inhibits homology-directed repair (HR) and may be involved specifically in NHEJ (non homologous end-joining), given the dependence of its recruitment on the NHEJ factor Ku70/80 (Hong et al., 2008). In contrast, the PRC1 subunit BMI1 *(*B lymphoma Mo-MLV insertion region 1 homolog) stimulates HR (Ginjala et al., 2011), and its accumulation at damage sites depends on the early DSB repair factor NBS1 (Nijmegen breakage syndrome) rather than on Ku (Ismail et al., 2010). Thus, an exciting possibility is that PRC1 and PRC2, in combination with their accessory/regulatory factors, participate in the choice between the two main DSB repair pathways, i.e. HR versus NHEJ. The mechanism whereby PcG proteins promote DSB repair is unclear but it may relate to their contribution to the accumulation of DDR factors, as found for BMI1 in the early

recruitment of BRCA1 and 53BP1 (Ismail et al., 2010; Figure 3). Other histone modifying enzymes, as exemplified by HDAC1 and 2, generally considered as repressive factors, also accumulate at damage sites where they stimulate DSB repair by NHEJ (Miller et al., 2010). Whether these enzymes can be recruited on their own or as part of a larger complex as shown for HDAC1 within the NuRD complex (nucleosome remodeling deacetylase; Polo et al., 2010) remains to be established. We summarize in Figure 3 how the different repressive factors described above get targeted to sites of DNA damage. Finally, it is important to highlight here that most of them (if not all) may also potentially participate in the DDR to promote transcription inhibition at sites of DNA damage (O'Hagan et al., 2008; Chou et al., 2010; Miller et al., 2010), to avoid negative interference between active transcription and the DDR machineries.

Together, the unexpected positive contribution of heterochromatin proteins to the DDR as described in this section shows that heterochromatin-associated factors are not merely "obstacles" interfering with specific repair or signaling pathways and that chromatin relaxation is only one aspect of the response to DNA damage.

Our understanding of the crosstalks between heterochromatin proteins, modifications on particular histone variants and DDR factors is only beginning, and future work should help to provide an integrated view in the whole nucleus for the dynamics of these proteins in space and time.

Conclusions & future challenges

In conclusion, chromatin should be considered as an integral player in the DDR, at the level of its nucleosome components, the histone variants, as well as the level of other

chromatin proteins involved in heterochromatin. In this picture, chromatin factors would act as a dynamic "platform" that promotes the assembly and activity of signaling and repair machineries by preparing regions around the site of damage. Notably, DDR and chromatin factors are not functioning as separate entities but in an integrated fashion. Together, this analysis leads us to revise the "*Access Repair Restore (ARR)"* model (Smerdon, 1991; Green and Almouzni, 2002; Groth et al., 2007b) by reconsidering the mechanistic aspects of each individual step (Figure 4).

The "*access*" step, as initially proposed, implies that proteins are stripped of chromatin to expose DNA lesions to repair machineries, by nucleosome sliding away from damage sites and/or eviction of old histones (Figure 1). However, we have learnt that chromatin proteins are also recruited at these early steps of the DDR. Thus, to account both for the "in" and "out" dynamics of chromatin components operating with the concomitant recruitment of DDR factors, we propose to refer to a "prime" step. This priming step would enable to define a competent DDR region, where DNA damage signaling and repair can take place, with repair occurring at the damage sites and signaling spreading away (Figures 1 and 4; for review see Lukas et al., 2011; Polo and Jackson, 2011). This preparation step, depending on the context (cell cycle, location, type of damage), may determine the choice of a specific DNA repair pathway and ensure a proper coordination of the DDR with other nuclear functions, for example to inhibit transcription or delay replication.

In the "repair" step, it is important to integrate signaling pathways in addition to the crosstalks with chromatin dynamics. Some degree of overlap likely exists between "prime" and "repair", and these steps cannot be regarded simply as sequential. For instance, if we consider DNA repair in heterochromatin domains (Figure 2), we

realize that early repair/ signaling steps can already take place in compact chromatin regions.

Similarly, the "restore" step cannot be considered in isolation without overlapping with repair (Figure 4), since histone eviction could be accompanied with deposition in areas surrounding the damage before completion of DNA repair. These dynamics require histone chaperones to promote the re-establishment of chromatin organization, which involves not only a simple recycling of parental histones, but also incorporation of new histones. Thus, the nucleosomal organization re-established within the damaged area will not (at least transiently) reproduce parental chromatin. The incorporation of new histones bearing typical PTM patterns and distinct variants may alter the functional properties of the restored region (Figure 4). Indeed, nucleosomes incorporating different variants and PTMs show specific structural and functional properties (Dechassa et al., 2011). In addition to these changes, nucleosome positioning may also be altered upon chromatin restoration since nucleosomes are not necessarily put back at the same place. In light of these data, it will be important to consider the contribution of the DDR to histone turnover in the genome, and not just transcription and replication-mediated histone dynamics. It will also be particularly interesting to determine the proportion of new versus old histones in the restored regions by considering specific loci (e.g. transcriptionally active vs. inactive). Is there a threshold in this proportion beyond which maintenance of the pre-existing epigenetic state is compromised? Another key issue for chromatin restoration after repair is whether it can leave a "damage imprint" as a record of the exposure to DNA damage (Figure 4). To assess the impact of such an imprint on the cell fate, it will be important to further determine if these are transient or longer-term changes that can be inherited through cell generations. Most intriguing is whether this imprint, which in

some cases may not be visible at the DNA level, could make the cell react faster or differently upon a second exposure. This parameter may be important for our appreciation of the actual sensitivity to radiation exposure. We thus hope that progress in integrating the chromatin dimension in the DDR will help to understand how DNA damage may impact on both genomic and epigenomic stability.

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Histone variants		Factors involved in dynamics		Responses to DNA damage		Key ref.
Name	**% of total**	**Histone chaperones**	**Remodeling complexes**	**Mobilization/ modification**	**Function in the DDR**	
H2A variants						
H2A.1/H2A.2	Major forms	FACT (*Hs*)	Tip60 (*Hs*)	Modified by acetylation, ubiquitylation (*Hs*)	Recruitment of DDR factors (*Hs*)	*
H2A.X	5-20%	FACT *(Hs)*	Tip60 *(Hs, Dm)* INO80 *(Sc)*	Modified by phosphorylation, acetylation, ubiquitylation	Promotes DNA damage signaling (*Sc* to *Hs*)	[1-4]
H2A.Z	10%	NAP-1, Chz1 *(Sc)*	SWR1, INO80 *(Sc)*	Deposited at DSBs, acetylated (*Sc*)	Controls DNA damage signaling, promotes DSB resection (*Sc*)	[5-7]
macroH2A 1.1	up to 3%[#]	APLF *(Hs)*	n.d.	Macrodomain recruited to DNA damage via PAR binding (*Hs*)	Potential role in chromatin compaction at damage sites (*Hs*)	[8,9]
macroH2A 1.2		n.d.	n.d.	n.d.	n.d.	*
H2A.Bbd	n.d.	n.d.	n.d.	n.d.	n.d.	*
TH2A H2AL1/2/3	Testis specific	n.d.	n.d.	n.d.	n.d.	*
H3 variants						
H3.1	50%	CAF-1 (*Sc* to *Hs*)	n.d.	Newly synthezised histones deposited at damage sites (*Hs*)	Promotes checkpoint termination (*Sc*)	[10, 11]
H3.2	35%	n.d.	n.d.	n.d.	n.d.	*
H3.3	15%	HIRA complex (*Sc* to *Hs*) DAXX (*Mm*) DEK (*Mm*)	ATRX (*Mm*)	n.d.	n.d.	*
H3.4 H3.5	Testis specific	n.d.	n.d.	n.d.	n.d.	*
CenH3	n.d.	HJURP *(Hs)*	n.d.	Recruited to DSBs (*Hs*)	HR?, sensitivity to radiation (*Hs*)	[12, 13]
H3.X/H3.Y	n.d.	n.d.	n.d.	n.d.	n.d.	*

Table 1: H2A and H3 variant dynamics and functions in the DDR

Sc : Saccharomyces cerevisiae, *Dm* : Drosophila melanogaster, *Mm* : Mus musculus, *Hs*: Homo sapiens ; PAR : Poly(ADP-Ribose) ; HR : Homologous Recombination ; DSBs: DNA double-strand breaks ; % of total is indicated for human cells ; [#] : in differentiated cells ; n.d. : not determined

Key references:

1-4: (Rogakou et al., 1998; Kusch et al., 2004; Papamichos-Chronakis et al., 2006; Heo et al., 2008)

5-7: (Papamichos-Chronakis et al., 2006; Kalocsay et al., 2009; Papamichos-Chronakis et al., 2011)

8,9: (Timinszky et al., 2009; Mehrotra et al., 2011)

10,11: (Polo et al., 2006; Kim and Haber, 2009)

12,13: (Kato et al., 2007; Zeitlin et al., 2009)

* : (Talbert and Henikoff, 2010) for a general review on histone variants

Figure legends

Figure 1: Dynamics of H2A and H3 variants in response to DNA damage

This scheme highlights how the dynamics of H2A (left column) and H3 variants (right column) connect to the response to DNA double-strand breaks (DSBs), in particular to DSB signaling. First, phosphorylation of the H2A variant H2A.X by DDR kinases upon DSB detection initiates the signaling cascade (Signal on, green), and also contributes to inhibiting the H2A.X/H2A exchange mediated by the histone chaperone FACT in mammals. Concomitantly, parental histones are displaced from the damaged site by nucleosome sliding and/or eviction. Bidirectional spreading of γH2A.X away from the site of damage delineates a DDR competent region within potential boundaries as indicated by the question mark. Restoration of nucleosomal organization after repair involves histone chaperones (purple) and chromatin remodelers (blue) to promote potential parental histone recycling as well as new histone deposition (e.g. H3.1 deposition) and histone variant exchange (depicted here with H2A.X/H2A.Z exchange regulated by the opposite activities of SWR1/TIP60 and INO80 remodeling complexes). While new H3.1 histones deposited by CAF-1 in a repair synthesis coupled manner represent a source of histones, other H3 variants (including CenH3) could be provided independently of repair synthesis to fill potential gaps of "naked" DNA exposed upon parental histone displacement. Finally, nucleosome restoration and γH2A.X exchange contribute to turn off the DNA damage checkpoint (Signal off, red). #: H2A.X is also modified by ubiquitylation and acetylation in response to damage. *: any H2A or H3 variant.

Figure 2: Model for the response to DSBs within heterochromatin domains in higher eukaryotes. On the top left, we represent a mouse somatic cell**,** with HP1-enriched heterochromatin domains corresponding to chromocenters (in pink). A zoom on a single HP1-domain shows: (1) DNA damage at a nucleosomal level within the domain; (2) the early DDR steps taking place within the domain: phosphorylation of γH2AX (green hexagon) recognized by MDC1 (green hemicircle), and the recruitment of early repair factors (orange ovals representing RPA, XRCC1 and other early repair proteins). At this stage, the DDR is blocked (as depicted by a black cross over the signaling arrow): neither recruitment of late repair factors nor the spreading of DNA damage signaling occurs within the HP1-domain. (3) The subsequent expansion of the heterochromatin domain and extrusion of damaged DNA to the periphery of the domain enables the final DDR steps, including spreading of DNA damage signaling (green arrows) and completion of DNA repair (yellow ovals representing the recruitment of the repair protein RAD51).

Figure 3: The active role of heterochromatin proteins in the DDR. Several proteins known as repressive factors and/or components of heterochromatin (pink), including HP1 and its interacting partner KAP-1, HDAC1/2 and Polycomb group proteins (PcG) are actively recruited to DSBs (top arrows). This recruitment is aided by histone chaperones (CAF-1), chromatin remodelers (NuRD), specific DDR factors (Ku and NBS1, purple) and possibly other factors (others). Core and accessory subunits of Polycomb repressive complexes (PRC) found at damage sites include BMI1, Mel-18 and RING1 for PRC1 and EZH2, SUZ12, PHF1 and SIRT1 for PRC2. Here, we highlight BMI1 and PHF1 for their reported roles in signaling and repair. In general, the recruitment of heterochromatin factors promotes DNA damage signaling

events including histone modifications (H2A and H2A.X ubiquitylation), recruitment of checkpoint mediators (53BP1 and BRCA1) and impacts on DSB repair pathways (NHEJ and HR) (functional outcomes represented by bottom arrows). In the case of HP1, a concomitant release from its binding to H3K9me3 within heterochromatin takes place, which may contribute to increase the pool of HP1 available for recruitment to DNA damage sites.

Figure 4: A Prime-Repair-Restore model. In this model we rename the "Access" step "Prime" to account for both the positive and negative contribution of chromatin components (heterochromatin proteins, histones) during early steps of the DDR. A chromatin "toolbox" (blue), comprising histone chaperones, chromatin remodelers and modifiers, feeds into each step as indicated with the blue arrows. The temporal integration of the three steps is shown by the overlap between the three corresponding boxes (pink). The DDR compartment represents the competent region where both repair and signaling amplification are coordinated. New histone deposition and histone variant exchange occur during the restoration step at the nucleosome level. This may lead to an altered chromatin landscape as shown on the zoomed images with a typical parental nucleosome represented on the top (blue and white, marked with a parental PTM in pale blue) and a new nucleosome (green and black with a new PTM in yellow). The extent of the restored chromatin patch remains to be determined. The outcome of this restore process may leave a "damage imprint", i.e. a mark on chromatin, not necessarily visible on the DNA, that could have long-term implications on genomic/epigenomic stability and plasticity.

Figure 1

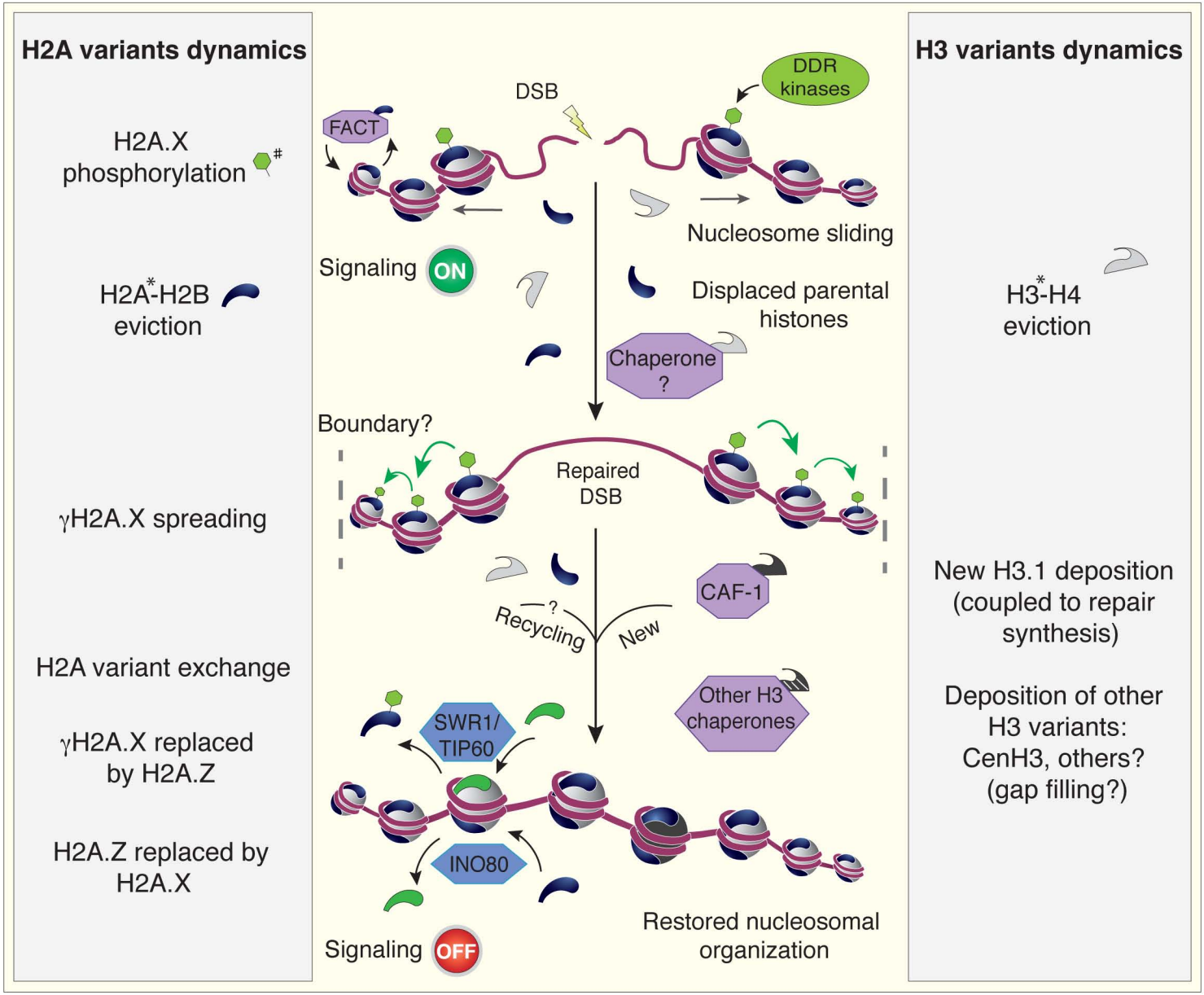

H2A variants dynamics
H2A.X phosphorylation #
H2A*-H2B eviction
γH2A.X spreading
H2A variant exchange
γH2A.X replaced by H2A.Z
H2A.Z replaced by H2A.X
DSB
DDR kinases
FACT
Nucleosome sliding
Signaling ON
Displaced parental histones
Chaperone ?
Boundary?
Repaired DSB
CAF-1
Recycling ?
New
Other H3 chaperones
SWR1/ TIP60
INO80
Signaling OFF
Restored nucleosomal organization
H3 variants dynamics
H3*-H4 eviction
New H3.1 deposition (coupled to repair synthesis)
Deposition of other H3 variants: CenH3, others? (gap filling?)

Figure 2

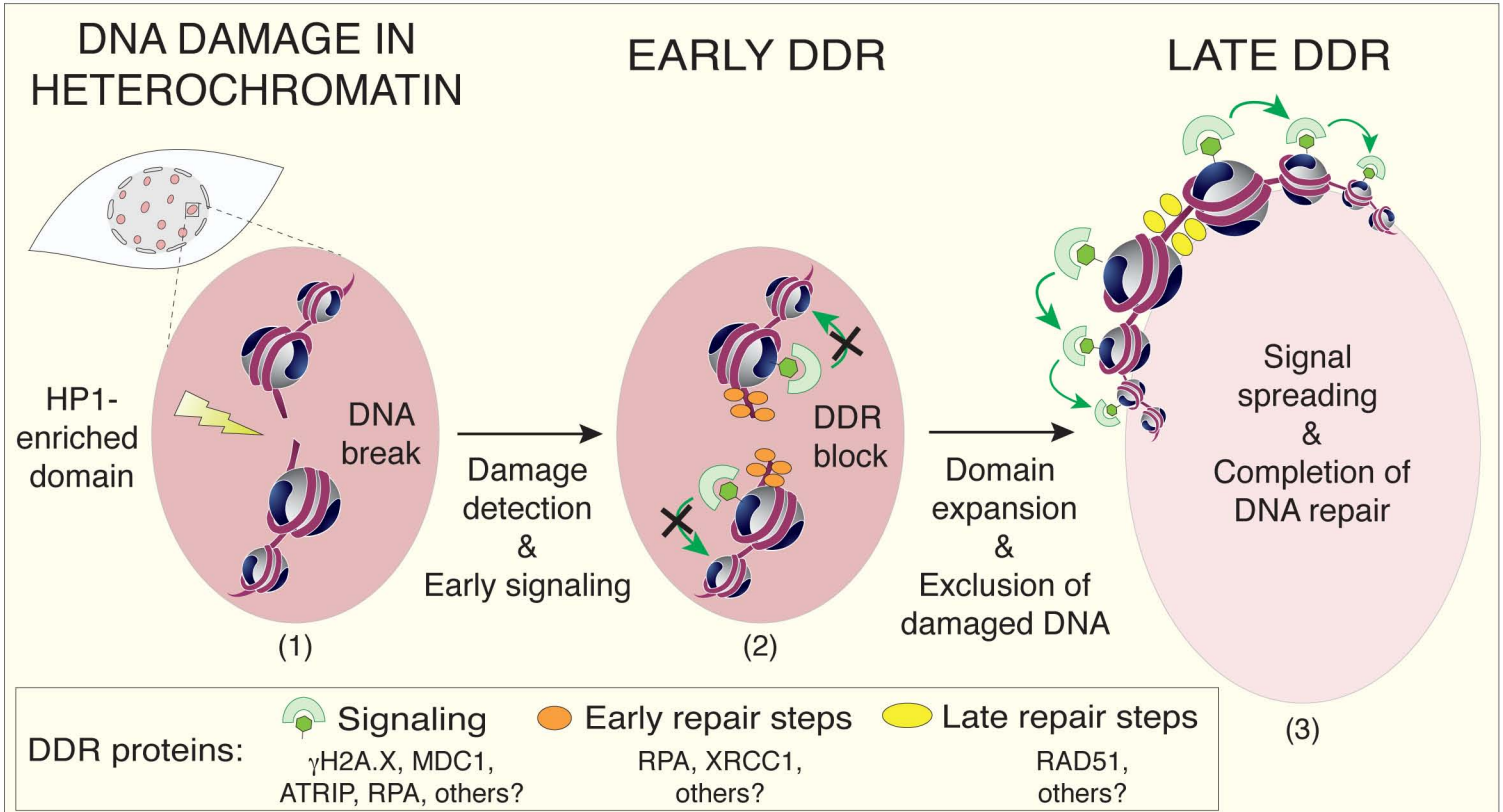

DNA DAMAGE IN HETEROCHROMATIN
EARLY DDR
LATE DDR
HP1-enriched domain
DNA break
(1)
Damage detection & Early signaling
DDR block
(2)
Domain expansion & Exclusion of damaged DNA
Signal spreading & Completion of DNA repair
(3)
DDR proteins:
Signaling
γH2A.X, MDC1, ATRIP, RPA, others?
Early repair steps
RPA, XRCC1, others?
Late repair steps
RAD51, others?

Figure 3

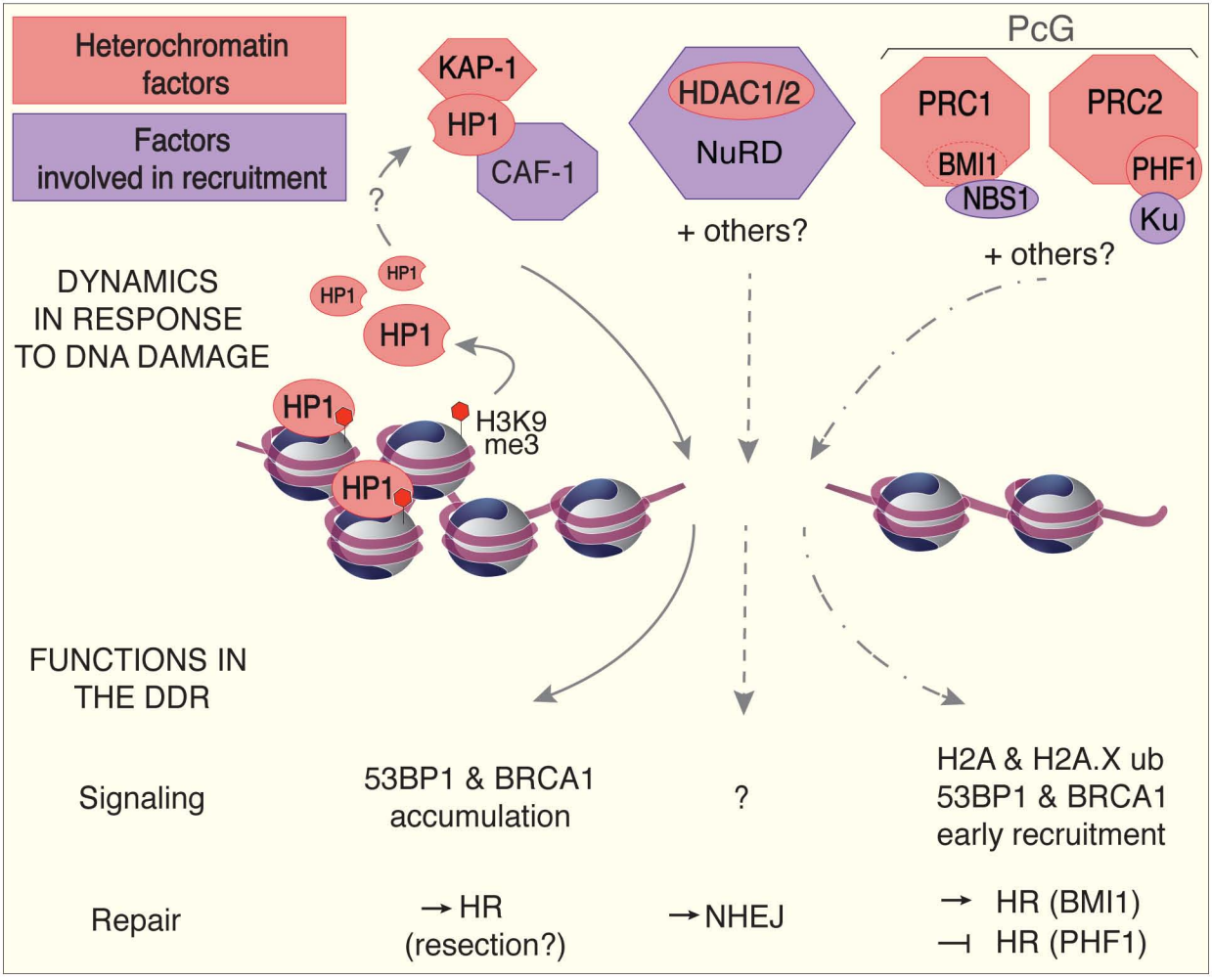
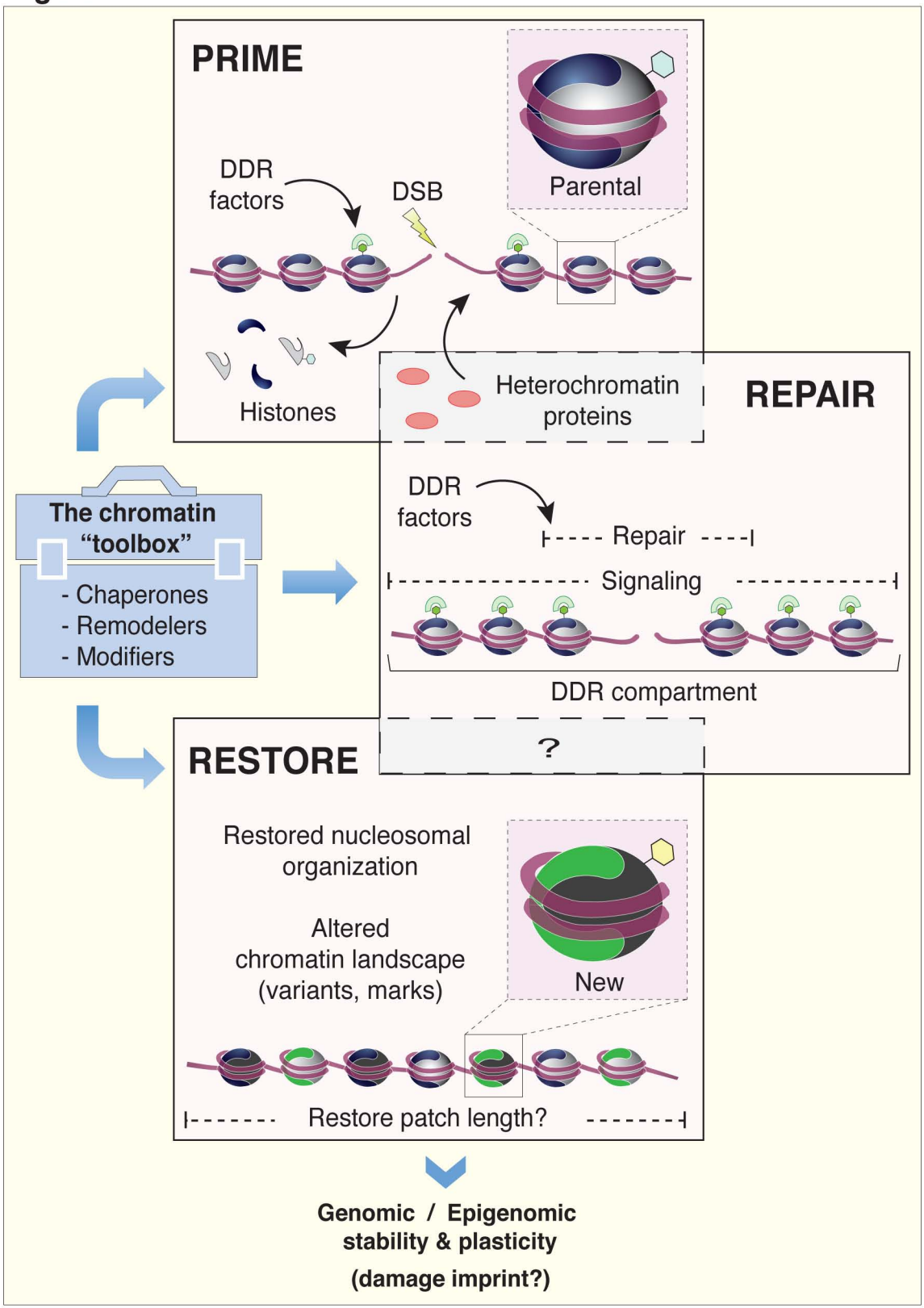

Heterochromatin factors
Factors involved in recruitment
KAP-1
HP1
CAF-1
?
HDAC1/2
NuRD
+ others?
PcG
PRC1
BMI1
NBS1
PRC2
PHF1
Ku
+ others?
DYNAMICS IN RESPONSE TO DNA DAMAGE
HP1
HP1
HP1
HP1
HP1
H3K9 me3
FUNCTIONS IN THE DDR
Signaling
53BP1 & BRCA1 accumulation
?
H2A & H2A.X ub
53BP1 & BRCA1
early recruitment
Repair
→ HR
(resection?)
→ NHEJ
→ HR (BMI1)
⊣ HR (PHF1)

Figure 4


PRIME
DDR factors
DSB
Parental
Histones
Heterochromatin proteins
REPAIR
The chromatin "toolbox"
- Chaperones
- Remodelers
- Modifiers
DDR factors
Repair
Signaling
DDR compartment
?
RESTORE
Restored nucleosomal organization
Altered chromatin landscape (variants, marks)
New
Restore patch length?
Genomic / Epigenomic stability & plasticity
(damage imprint?)