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Conversations between Chromatin Modifications and DNA Double Strand Break Repair: A Commentary

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When DNA damage arises in a eukaryotic genome, the repair machinery acts on a substrate that is packaged into chromatin. Over the past decade, we have begun to appreciate the importance of this distinction. A series of reviews in this issue examines how the DNA double-strand break (DSB) repair sensing, signaling, and repair machinery accesses and exploits a chromatinized DNA substrate to orchestrate DSB repair. There are two fundamental questions that are addressed throughout these reviews. What physical constraints does chromatin structure place on DSB repair? How is chromatin used as a signaling platform in the DNA damage response? These questions are closely related because processes that control chromatin structure can also control signaling. For example, the acetylation of histone H4 on lysine 16 is both a switch between the 10 nm and 30 nm chromatin fiber structures[1] and an inhibitor of 53BP1 binding to chromatin surrounding the DSB [2, 3]. It is important to understand both chromatin structure and DSB signaling in order to understand the regulation of DSB repair. Collectively, the reviews in this issue bring us up to date on these important areas that define the interface between chromatin structure and DSB repair biochemistry.

If the packaging of DNA into higher order chromatin structures beyond that of the nucleosome represents a physical barrier to DSB repair, mitotic chromosomes would be expected to present the greatest accessibility challenges. It has long been known that cells are most sensitive to radiation when they are exposed during mitosis[4], which one would predict if accessibility constrained DSB repair. Until recently, mitosis was largely overlooked in the study of DSB repair but the details are now beginning to emerge. Surprisingly, accessibility does not appear to limit DSB sensing and signaling in mitosis. As Van Vugt reviews in this issue, the emerging story is one of biochemical regulation of the signaling machinery rather than one of chromatin barriers that inhibit the repair of DSBs. Remarkably, the initial recognition of the DNA break by the MRN complex as well as the phosphorylation of histone H2AX and the generation of H2AX foci remains robust during mitosis. Homologous recombination repair (HR) does not occur during mitosis but there is evidence that some end resection can take place. This finding reveals that sensors can access DSBs that are located within chromatin in its most compact state; i.e. during mitosis. The HR pathway, however, is specifically impaired for reasons that are still incompletely understood. Additionally, evidence of end resection suggests that nucleosome remodeling may also be taking place near the DSB.

Although chromatin may not prevent all DSB repair in mitosis, DSB repair signaling is altered and HR suppressed during this phase of the cell cycle [5-7]. Van Vugt discusses emerging data on the primacy of retaining active cdks and proceeding through cell division. There remain many unanswered questions such as whether or not some end-joining repair takes place within mitotic chromosomes and if the break is not repaired in mitosis, why is it important to initiate the signaling in mitosis? Since the broken DNA is typically not lost in mitosis, but repaired in the subsequent interphase, why are DSBs arising during mitosis more toxic than those arising in interphase?

In interphase, the repetitive DNA surrounding the centromeres, termed pericentric heterochromatin, is typically the most compact genomic DNA. Although heterochromatin boundaries can block the spreading of the DSB signaling machinery along the chromatin, it appears that DSBs located within heterochromatin can be rapidly located and signaling initiated (see Chiolo et al., in this issue). Thus, the DNA damage sensing and early processing machinery does not appear to be physically excluded from mitotic chromosomes or interphase heterochromatin.

While heterochromatin and mitotic chromosome condensation may not pose a significant barrier to the initial recognition and processing of the DSB, nucleosome removal or displacement is clearly required for DSB repair to proceed. The removal or displacement of nucleosomes is an activity that is attributed to ATP-dependent chromatin remodeling complexes. These enzymes use ATP to dissociate nucleosome subunits, such as H2A-H2B dimers, to displace nucleosomes, or to translocate the DNA sequence relative to the nucleosome. These activities are particularly well understood in budding yeast, where the Swi/Snf and Ino80 complexes have both been shown to respond to DNA damage[8-10]. The contribution of ATP-dependent chromatin remodeling complexes is reviewed by Stanley et al. These authors provide an overview of the known roles of ATP-dependent chromatin remodeling enzymes in DNA damage signaling and repair with an emphasis on the more poorly characterized CHD family of proteins. CHD proteins are commonly found in complexes with HDACs 1 and 2 and contain tandem chromo and PHD domains, both of which bind to methylated histones. Interestingly, it is the displacement, rather than recruitment, of CHD3 that appears to be important for DSB repair. CHD3 can interact with sumoylated KAP1 and is retained in heterochromatin, where it functions to promote the heterochromatin state. Upon ATM activation and KAP1 phosphorylation, CHD3 is displaced, potentially enabling chromatin decondensation. Based on what we have learned about chromatin compaction and its impact on the accessibility of damaged DNA, it will be important to resolve whether displacement of CHD3 is required for heterochromatin formation/maintenance or whether CHD3 prevents the removal of one or more nucleosomes immediately surrounding the break site.

Although the accessibility and initial processing of the DSB appears to be able to accommodate the extremes in structure that chromatin can present, DSBs are typically only found in the interior of heterochromatin environment during the first few minutes of DSB repair[11, 12]. At later time points, the foci that form around DSBs localize to the surface of heterochromatin, indicating that they must somehow move from the interior to the surface of these domains. In the absence of DNA damage, the movement of both euchromatin and heterochromatin is constrained within the interphase nucleus. The diffusion of individual loci is typically confined to about 0.5 μm in each lateral direction (reviewed in [13]). Consequently, assembly and repair at most DNA breaks in mammalian cells occurs at the site of the break by recruiting complexes from the nucleoplasm. In the case of a DSB within the interior of heterochromatin, however, it appears to be important for the DSBs to move away from the heterochromatin environment in order for DSB repair to be completed. Interestingly, the movement of DNA double-strand breaks may not be describable as a

single phenomenon. Rather, Chiolo and colleagues discuss evidence that there are both mobile and immobile phases for DSBs with a particular emphasis on the movement of DSBs that arise in the interior of heterochromatin. As both Stanley et al., and Chiolo et al., discuss, there is evidence that the heterochromatin environment is refractory to the completion of DSB repair. It appears that, while the initial processing and signaling is insensitive to chromatin structure, later events do require greater accessibility and space to fully process repair of DSBs.

Chromatin structure is intimately linked to the presence of specific histone tail modifications. This observation has fostered the development of the concept that post-translational modifications on histone tails represent a signaling platform to control biological function on chromatin. The histone code hypothesis originated from the idea that histone modifications could be read to direct execution of biological processes[14]. This prediction requires the collective existence of “writers, readers, and erasers” of a putative code in the form of enzymatic activities and binding domains that specifically recognize single and combinatorial histone modifications. A causal relationship is particularly well supported by abundant ChIP-seq and genetic data. These show strong correlations between specific modifications and different chromatin domains while organisms harboring mutations in chromatin “writers, readers, and erasers” often display dysfunction in chromatin structure coincident with alterations in transcription and genome integrity control[15, 16].

The growing number of repair proteins and insights into their manner of DNA damage recognition attests to the complex nature of recognition events at DSBs within chromatin and how post-translational modifications play an integral role in establishing the hierarchy of the DSB response. As such, DNA damage recognition represents a striking example of how the language of chromatin modification converges on DNA repair processes. Indeed, the presence of large DNA repair protein foci that assemble around DSBs represents a convenient measure of chromatin contributions to DNA damage responses. Defining the biochemical requirements for their formation has led to the establishment of a hierarchical network of post-translational modifications on chromatin that are deposited, read and removed in a temporally and spatially distinct process during the DNA damage response (DDR)[17-19].

Xie and Scully describe this hierarchy from a functional perspective using the histone H2A variant, H2A.X as a cornerstone of the chromatin response to DSBs. Although both HR and NHEJ are impaired in H2A.X null cells, they remind us that H2A.X is not an essential gene and that the assembly of stable foci is not absolutely required for all DSB repair. On the other hand, Mermershtain and Glover take a structural biology approach to this signaling axis, describing molecular recognition events that enable a step-wise build up of DNA repair foci. H2A.X represents approximately 1-10% of all H2A species in the cell. Phosphorylation by PI3 kinase like kinases ATM, ATR and DNA-PK initiate much of the chromatin response that is required for foci formation[17, 18]. Recognition of H2A.X by the MDC1 tandem BRCA1 C-terminal repeat (BRCT) motifs is necessary for a tightly linked ubiquitination and SUMOylation cascade that enables subsequent assembly of 53BP1, BRCA1 and many other repair proteins at DSB chromatin. These events are largely driven by the classic paradigm for chromatin biology of “readers, writers, and erasers.” PIKKs and E3 ubiquitin ligases RNF8 and RNF168 write the code that directs assembly of reader proteins MDC1, RAP80, 53BP1 and others to chromatin along DSBs. RAP80 binds most efficiently to lysine63-linked ubiquitin chains[20], while 53BP1 uses its tandem Tudor domains to bind histone H4 dimethylated at lysine 20[21]. Interestingly, both 53BP1 and RAP80 show combinatorial reading function for several of these proteins. 53BP1 recognizes dual marks of H4K20me2 and H2A ubiquitinated at K15[22], while H4K16 acetylation reduces 53BP1 affinity for

K20me2 when present on the same histone tail[23]. RAP80 also displays combinatorial reading as it binds hybrid SUMO2,3-K63-Ub chains with approximately 80-fold higher affinity than K63-Ub alone[24]. It is likely that many more examples of combinatorial signals will emerge in the context of DNA repair.

It has become increasingly clear that while some chromatin modifications are DNA damage inducible, DSB recruitment events are also influenced by the pre-existing chromatin structure of where a break occurs[25, 26]. This raises the possibility of non-mutually exclusive models for spreading of the DNA damage response. On the one hand, spreading could simply be a consequence of the availability of writers of DNA damage response modifications, while on the other hand, intrinsic structural features of pre-existing chromatin structure could influence spreading of the DSB signals along chromatin. Evidence for both scenarios exists[27, 28]. Notably, H2AX spread is different between heterochromatin and euchromatin. It has also been proposed that heterochromatin and euchromatin have vastly different repair kinetics and dependencies on ATM kinase signals and repair mechanisms[25, 26]. A second example is that approximately 80-90% of histone H4 species contains dimethylation at lysine 20 due to combined actions of the PR-Set7 H4K20 monomethyltransferase and the SUVK4-20 dimethyltransferases[29, 30]. This mark is a requirement for 53BP1 chromatin association, anchoring it through the 53BP1 Tudor domains[21].

A third example of interactions between the pre-existing chromatin structure and the DDR is acetylation. Histone acetylation has been historically linked with transcriptionally active genes in euchromatin and promotes chromatin decondensation in vitro[31, 32]. A growing body of literature suggests that acetylation is dynamically regulated at DSB chromatin and influences both early and late stages of the DDR. Indeed, the spread of H2AX is dramatically increased when the acetyl-lysine binding protein Brd4 is diminished or absent[28]. Gong and Miller provide a sophisticated view of how the kinetics of deacetylation and acetylation affect DSB repair and which DSB repair mechanism, HR or NHEJ, when repair at a DSB ensues. Specifically, histones are deacetylated by rapid recruitment of the class I histone deacetylases HDAC1 and HDAC2 within minutes of DSB induction[33]. Deacetylated chromatin at the early stage of response becomes re-acetylated over a period of hours by the combined action of numerous lysine acetyltransferase enzymes, which include p300/CBP, TIP60, and Mof[19, 34, 35]. The dynamics of acetylation closely mirror DSB foci formation by powerful regulators of DNA repair mechanisms. Early waves of deacetylation are required for NHEJ and are coincident with 53BP1 foci formation, which limits the extent of DNA end resection by endo- and exo-nucleases that precede engagement by the HR machinery.

The cellular response to DSBs involves a remarkable diversity of proteins and protein modifications with chromatin playing a central role beginning with the phosphorylation of histone H2AX. Recent studies reveal a surprisingly minimal role for the pre-existing packaging of chromatin as a physical barrier to the sensing of DNA double-strand breaks, although the expansion of H2AX can be limited by boundaries between euchromatin and heterochromatin. Even DSBs formed in the interior of densely packaged structures such as pericentric heterochromatin and mitotic chromosomes appear to be very rapidly detected. Upon detection, a diverse array of chromatin remodeling and modifying machinery is able to remodel the underlying chromatin structure such that chromatin packaging does not prevent the assembly of signaling and repair complexes onto the chromatin. This involves chromatin remodeling machinery that repositions, compositionally remodels, or displaces nucleosomes and histone posttranslational modifications that regulate chromatin packaging and function to regulate the signaling that takes place on the chromatin template. At its extreme, this can involve the physical movement of DSBs from the interior to the exterior of dense

heterochromatin domains, demonstrating the remarkable capacity of the DSB repair machinery to circumvent the constraints of the pre-existing chromatin environment.

There remain significant outstanding questions regarding the interface between chromatin and DSB signaling. Deep sequencing of DNA sequences involved in chromatin-chromatin interactions, based on the chromosome conformation capture (3C) assay, has revealed that chromatin is organized into topologically associated domains (TADs) that have a high probability of interacting with each other inside the nucleus but a lower probability of interacting with sequences outside of their TAD [36]. These interactions are directional, implying the existence of a chromatin structure that promotes these interactions. Their megabase and submegabase pair size is very similar to estimates for the amount of DNA in H2AX foci. Do these TADs define the domains of chromatin that are modified by the DSB signaling machinery? Is the structure that these domains adopt, which significantly reduces their interactions with surrounding sequences, important in the regulation of the spreading of the signaling from the DSB? The involvement of Brd4 and cohesin in limiting the spread of H2AX could be related to such a mechanism. Alternatively, is the determinant of this spreading dictated solely by controlling signaling? Evidence that RNF168 is limiting in DDR signaling and that its cellular concentration is tightly regulated suggest that there may be a simple biochemical explanation. Clearly, there is much more to learn.

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