ATM Breaks into Heterochromatin

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Heterochromatin is refractory to DNA transactions, including repair. In a recent issue of *Molecular Cell*, Goodarzi et al. (2008) reveal how the central transducing kinase of the DNA damage response relieves this natural barrier by increasing heterochromatic DNA accessibility.

Naked DNA does not exist outside of the biochemist's bench-top. Within cells, DNA is packaged around protein complexes that modulate many physiological processes, including transcription, replication, cohesion, and cell division. Similarly, the detection, signaling, and repair of DNA damage takes place within the context of this proteinaceous-embedded DNA called chromatin. Octamers of acidic proteins known as histones make up the basic unit of chromatin, referred to as the nucleosome. Nucleosomes themselves are also differentially segregated in space, and a bird's eye view of the nucleus clearly illustrates the heterogeneity of chromatin within a single cell (Figure 1). A simple classification can be made according to protein density. Protein-dense regions make up what is defined as heterochromatin. This tightly packed DNA is for the most part genetically inactive. The remaining DNA comprises euchromatin, and these open regions are associated with a higher transcriptional activity.

Heterochromatin is required for numerous cellular processes, including chromosome segregation (the ability of cells to divide properly and pass on their genetic material), dosage compensation in females (transcriptional inactivation of one of the two X chromosomes), and meiotic progression in males (formation of the XY body during spermatogenesis). However, although it is essential for life, chromatin packaging is a barrier to the detection and repair of DNA damage (for a comprehensive review, see Downs et al., 2007). Perhaps the first indication that DNA repair efficiency is not equally distributed throughout the nuclear space came from an electron microscopic study performed more than three decades ago (Harris et al., 1974). This work demonstrated that DNA repair synthesis following exposure to ultraviolet light or carcinogens was less efficient within heterochromatic regions relative to less compact regions. Since then, several studies have demonstrated that the location of a DNA double-strand break (DSB) within the nucleus, or within a chromosome, can influence DNA repair efficiency. Moreover, it appears that nature has evolved to preferentially generate physiological DNA damage within open chromatin rather than condensed regions. For example, heterochromatic regions such as centromeres or telomeres are rarely targets of self-inflicted DSB formation during meiotic recombination or antigen receptor rearrangements.

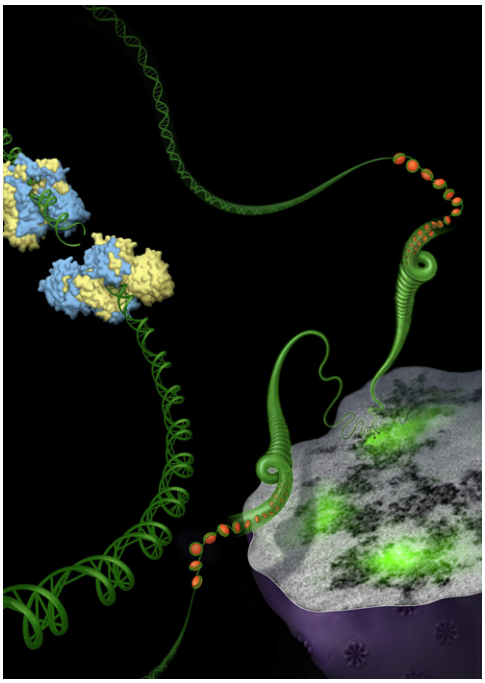

Figure 1. ATM-Mediated Phosphorylation of Chromatin Proteins Increases the Accessibility to DNA Breaks
This image shows an irradiated mouse thymocyte nucleus monitored at high resolution by electron microscopy (white signal on a dark background corresponds to densely packed chromatin). Phosphorylated histone H2AX (green fluorescence) maps the sites of DNA breaks. In response to this lesion, chromatin is remodeled by ATM (and other factors) to a more open configuration (darker areas in nucleus correspond to less condensed chromatin). This chromatin decondensation allows DNA repair proteins (modeled in blue/yellow) to bind and repair the damage. Illustration by Michael Kruhlak, Alan Hoofring, and A.N.

In contrast to programmed damage, ionizing radiation (IR) is thought to generate DNA damage throughout the genome. The question addressed by the current work from Penny Jeggo's group (Goodarzi et al., 2008) is how the DNA damage signaling pathways contend with lesions that happen to land within compact chromatin. The basis of the current study was an earlier observation from this group that ATM, one of the essential transducing kinases of the DNA damage response, is required for the repair of a subfraction (approximately 15%) of IR-induced DSBs (Riballo et al., 2004). The extreme IR hypersensitivity of ATM-deficient cells had traditionally been attributed to a fraction of DSBs that are not repaired efficiently; however, the nature and location of the unrepaired breaks was not defined until now. Goodarzi et al. (2008) noted that this residual level of unrepaired breaks was equivalent to the fraction of DNA that lies within heterochromatin. This finding led them to explore whether there was

a relationship between the breaks that were not being repaired in ATM-deficient cells and the complexity of the surrounding chromatin. Indeed, they provide biochemical and cell biological evidence that persistent breaks preferentially lie in close proximity to heterochromatin, suggesting that ATM plays a specialized role in repairing lesions found in compacted chromatin.

But how is ATM's chromatin remodeling activity linked to its role in DNA damage signaling? Given that ATM kinase activity is essential for its function in DNA repair (Riballo et al., 2004), Goodarzi et al. (2008) speculated that ATM could impact chromatin structure by phosphorylating proteins that are involved in chromatin organization. Previous work from the Shiloh laboratory established a role for an ATM effector, the transcriptional corepressor KAP-1 (also called TIF1β) in chromatin organization following DSB induction. They discovered that ATM phosphorylates KAP-1 at DSB sites and that phosphorylated KAP-1 rapidly migrates throughout chromatin, thereby promoting its relaxation (Ziv et al., 2006). Strikingly, Goodarzi and colleagues now show that increasing the chromatin accessibility via KAP-1 depletion can restore DNA repair competence in ATM-deficient cells. Similarly, the removal of other chromatin modulators, such as HP1 and histone deacetylase HDAC1/2, which normally promote chromatin compaction, bypasses the requirement for ATM in DSB repair. Furthermore, ATM-mediated phosphorylation of KAP-1 weakens its chromatin binding capacity. Although heterochromatin does not appear to be substantially dismantled, the changes might facilitate the relaxation of heterochromatin sufficiently to enhance the access of enzymes essential for end-joining (Figure 1). These findings are complementary to recent studies, demonstrating that chromatin relaxation enhances homologous recombination and ATR signaling (Murga et al., 2007).

It is likely that KAP-1 mobilization is just the tip of the iceberg and that several strategies have evolved to facilitate DNA repair at heterochromatin. For example, a recent high-resolution electron microscopic study showed local chromatin relaxation in the vicinity of a DSB (Kruhlak et al., 2006). This decondensation occurs independently of ATM but still requires active processes that metabolize ATP (Kruhlak et al., 2006). Another study showed ATM-independent, but Casein Kinase II-dependent, HP1 phosphorylation in response to DNA damage, an event that decreased the affinity of HP1 for chromatin (Ayoub et al., 2008). Thus, distinct but complementary mechanisms have emerged that remodel chromatin structure in a manner that increases the accessibility of underlying lesions in DNA.

Although ATM might play a role in increasing the accessibility to DNA breaks that arise in condensed regions, this finding does not preclude a function for the kinase in the repair of DNA damage embedded within more accessible, loosely packaged regions. ATM is required to repair a subset of breaks incurred during lymphocyte-specific V(D)J recombination and class switch recombination, but the barrier to the recombinases that cleave DNA is relieved by transcription-mediated chromatin decompaction during lymphocyte development (Downs et al., 2007; Dudley et al., 2005). ATM is also essential for repair during meiosis, and meiotic DSBs are thought to be generated within accessible chromatin domains. ATM-mediated phosphorylation might enhance the activity of repair enzymes, or enhance their retention at DSB sites. Finally, patients with the disease ataxia telangiectasia (caused by autosomal recessive mutations in the *ATM* gene) develop progressive degeneration of cerebellar Purkinje neurons. Importantly, in Purkinje cells, which are badly affected by lack of ATM, chromatin is highly euchromatic. Thus, the importance of ATM-mediated facilitation of DSB repair in heterochromatin could be different in various cell types. Regardless of whether ATM functions exclusively to promote repair at heterochromatin, the new data reported by Goodarzi et al. (2008) suggest novel therapeutic strategies that might potentiate DNA damage signaling and repair. Accordingly, the use of agents that decompact chromatin might restore DNA repair competence to patients with ataxia telangiectasia or related chromosome instability disorders.

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