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AUTHOR'S VIEW

A TAD closer to ATM

Francois Aymard[a,b] and Gaëlle Legube[a,b]

[a]LBCMCP, Center for Integrative Biology (CBI), Université de Toulouse, UT3, Toulouse, France; [b]LBCMCP, Center for Integrative Biology (CBI), CNRS, Toulouse, France

ABSTRACT
Ataxia telangiectasia mutated (ATM) has been known for decades as the main kinase mediating the DNA double-strand break response. Our recent findings suggest that its major role at the sites of breaks likely resides in its ability to modify both the local chromatin landscape and the global chromosome organization in order to promote repair accuracy.

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Ataxia telangiectasia (AT) is a severe human genetic disease that is caused by a mutation in the ataxia telangiectasia mutated (*ATM*) gene and is associated with neurodegeneration, predisposition to infection, and an increased risk of cancer. ATM belongs to the phosphoinositide 3-kinase (PI3K)-like protein kinase family involved in the DNA double-strand break (DSB) response in higher eukaryotes. Extensive studies have revealed that ATM exhibits a pervasive activity from local promotion of the detection and repair of DSBs to more global activation of checkpoints. However, AT patients, although completely devoid of ATM activity, are viable, indicating either a non-essential function of ATM for DSB repair or partial compensation by other kinases such as the DNA-dependent protein kinase (DNA-PKcs), which is a widely acceptable idea given the large overlap among all PI3K-like kinase substrates. Unfortunately, despite tremendous efforts to unravel the respective functions of these kinases during the DNA damage response (DDR), the overall picture remains blurry: first, it seems that the kinases regulate each other in a yet poorly characterized manner and, second, their roles have been studied following exposure to a wide range of DSB inducing agents, which induce clean *versus* dirty ends and act at different cell cycle phases and at various genomic positions, thus leading to contradictory results. In a recent report we made use of the DSB inducible via AsiSI (DIvA) system to investigate the respective functions of ATM and DNA-PKcs following the induction of hundreds of clean DSBs throughout the human genome.[1] Beyond our finding that DNA-PKcs inhibition indeed impairs repair at all DSBs irrespective of their localization on the genome, our results suggest that ATM should be considered as a master chromatin/chromosome organizer in response to DSBs.

Using a high-throughput genomic approach (ChIP-chip) we found that ATM is locally recruited to a region roughly spanning 2–10 kb. Once set, ATM catalyzes the phosphorylation of H2AX on the entire megabase chromatin domain; this holds true for all DSBs induced by *Asi*SI in different euchromatin contexts (intergenic regions, active genes, and inactive genes) and irrespective of the pathway used for repair (homologous recombination or non homologous end joining). The sharp contrast between the confined ATM distribution and the megabase-wide H2AX phosphorylation (known as γH2AX),[1] together with our previous observation that γH2AX domain boundaries coincide with topologically associated domain (TAD) boundaries[2] shed light on the mechanism by which ATM achieves the establishment of a γH2AX domain: nucleosomes likely get phosphorylated when brought to the proximity of ATM bound to DNA ends thanks to the dynamics of the chromatin fiber within a predefined TAD (Fig. 1). Notably, in yeast, Mec1 and to a lesser extent Tel1 (ataxia telangiectasia and Rad3-related [ATR] and ATM homologs respectively), are also able to phosphorylate nucleosomes located on other, undamaged, chromosomes spatially close to the DSB (i.e., when a DSB is induced near a centromere *trans* γH2AX spreading is observed on other, clustered, centromeres).[3] Whether ATM could enhance the dynamics of the chromatin within a TAD after the induction of a DSB, thereby further enhancing its ability to establish the gH2AX domain, remains an open question. Such an ATM-dependent increase in chromatin mobility would be in agreement with previous demonstrations of irradiation-induced foci (IRIF) mobility upon ATM inhibition,[4] and with its described function in the repair of DSBs induced in heterochromatin. Indeed, at those DSBs, ATM is required for repair in a manner that depends on its ability to relax heterochromatin.[5] Interestingly, our study revealed that upon inhibition of both ATM and DNA-PKcs, gH2AX domain establishment is partially rescued but on a narrower chromatin domain (~200 kb compared with 1–2 Mb in ATM proficient cells). One interpretation of these data may be that a third kinase compensates for the loss of ATM with respect to H2AX phosphorylation, but not for another yet

CONTACT Gaëlle Legube gaelle.legube@univ-tlse3.fr

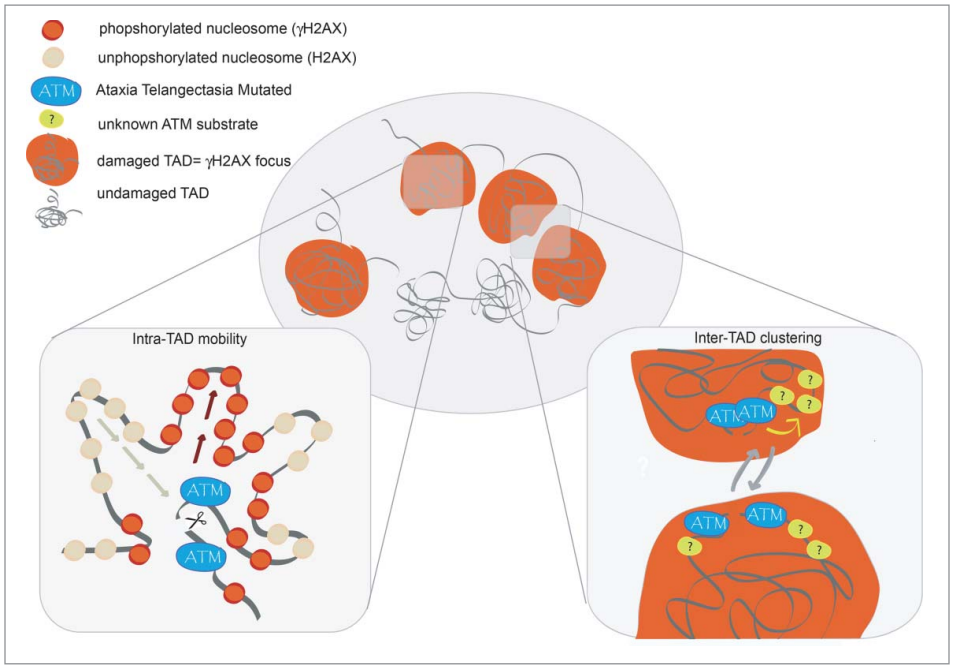

Figure 1. ATM-mediated H2 AX phosphorylation and DSB clustering. Upon damage detection, ataxia telangiectasia mutated (ATM) is recruited to a restricted region surrounding the double-strand break (DSB). H2AX-containing nucleosomes present within the damaged topologically associated domain (TAD, in red) are then phosphorylated, possibly as a result of local chromatin mobility that brings nucleosomes into the spatial vicinity of ATM. This leads to spreading of γH2AX through the entire damaged TAD. ATM may also phosphorylate other chromatin substrate(s) (in yellow) to locally enhance the mobility. This enhanced mobility could lead to increased H2AX phosphorylation within the TAD and favor DSB clustering.

unidentified substrate whose main function might be to enhance chromatin mobility. Interesting candidates for such a function include the cohesin complex since (i) the structural maintenance of chromosomes 1 (SMC1) subunit of the cohesin complex is a well-known substrate for ATM following damage, (ii) cohesin is a *bona fide* chromatin constituent that plays a role in chromosome architecture and chromatin stiffness, (iii) ATM-dependent phosphorylation of SMC1 modulates cohesin residence time on chromatin,[6] and (iv) cohesin regulates γH2AX enrichment around DSBs.[2]

Although further investigations are required to verify whether ATM promotes chromatin mobility locally around the DSB, our study also points toward a role of ATM in global chromosome reorganization within the nuclear space. Indeed, using high-resolution microscopy and live imaging we found that *Asi*SI-induced DSBs cluster within repair foci in an ATM-dependent manner (Fig. 1). Whether DSBs can coalesce in higher eukaryotes (as they do in yeast) has been the subject of strong controversy but recent ground-breaking studies indicate that such a mechanism could also exist in human cells. First, Greenberg's laboratory reported that DSBs induced near telomeres are mobile and cluster together in a Rad51-dependent manner.[7] Second, studies from Misteli's laboratory indicate that I-SceI–induced DSBs occasionally lead to translocations, a phenomenon that is preceded by the juxtaposition of the 2 DSBs.[8] Our findings generalized these observations and indicate that DSB clustering is probably more common than previously expected. However, it remains to be determined whether clustering ability is restricted to a subclass of breaks—in other words, whether this behavior depends on the genomic location of the break. Notably, possible mechanisms were recently brought to light by the laboratories of de Lange and Mullins, which respectively showed that microtubules and actin filaments contribute to DSB mobility and/or repair.[9,10]

This ability to bring distant DNA ends into close proximity seems paradoxical given the increased risk of translocation in those conditions. Strikingly, we found that although ATM is dispensable for DNA end rejoining, it promotes repair accuracy.[1] Thus, whether ATM-mediated DSB clustering promotes repair fidelity is an exciting hypothesis that deserves further investigation. While future work is required to determine the function and regulation of DSB clustering, we believe that this emerging role of ATM in local and global chromatin mobility should be taken into account in the etiology of ataxia telangiectasia.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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