Cell: Preview

ATM creates a veil of transcriptional silence

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Summary

The ATM kinase orchestrates diverse DNA damage responses. By putting together a fluorescence-based reporter that allows simultaneous monitoring of DNA damage responses as well as transcription in single cells, Greenberg and colleagues now uncovered a role of ATM in loading a DNA Double-strand break Induced Silencing in Cis (DISC) program.

Main Text

Emanating from a DNA double-strand break (DSB) is a plethora of post-translational protein modifications (PTMs) that spread and decorate megabase chromatin domains flanking the lesion. While these PTMs accumulate into microscopically visible foci that are generally believed to promote DNA repair and cell survival, the functional roles of many of these PTMs at the vicinity of DSBs remains to be determined; one of these being mono-ubiquitylated-H2A (uH2A). Stemming from previous studies that implicate uH2A in transcriptional silencing (Weake and Workman, 2008), Greenberg and colleagues designed an elegant reporter system to examine whether uH2A may also exert similar gene silencing activities at the damaged-modified chromatin (Shanbhag et al., 2010).

To do that, the authors borrowed a previously described transcriptional reporter (Janicki et al., 2004) and re-engineered it so that a defined DSB can be generated at a stretch of DNA sequence adjacent to the transcription unit. By employing fluorescence-based designs, the system made it possible to simultaneously observe, both qualitatively and quantitatively, nascent transcription, protein production, as well as DNA damage responses all at the single-cell level.

Introduction of DSBs in interphase chromatin not only disrupts its physical integrity, but has long been envisioned to interrupt numerous DNA transaction processes that take place at this dynamic structure. Whereas DSBs appear to inhibit DNA replication by preventing global origin firing and slowing the progression of local replication forks, it is not known whether and how these DNA lesions modulate local transcription. Now using this setup, Greenberg and colleagues addressed this

question by measuring transcriptional activities adjacent to the engineered DSB site. They found that transcriptional activities at the chromosomally integrated reporter were largely repressed when a DSB was introduced. What's more interesting was that this DSB-associated gene silencing response was only effective on chromatin regions proximal to the lesion, and did not affect transcription at a distance.

Calling this the DNA Double-strand break Induced Silencing in Cis (DISC) phenomenon, the authors went on to uncover a strict requirement for the ATM kinase in mediating, amongst others, this DNA damage response. Notably, DISC coincided with two hallmarks of transcriptional repression: RNAPII stalling (hypo-phosphorylation of RNAPII) and impaired transcription-associated chromatin decondensation, pointing to the idea that DISC effects through canonical transcription regulatory mechanisms. Prompted by the implicated role of mono-ubiquitylated-H2A (at Lysine 119) in transcription inactivation, the authors asked whether uH2A molecules that contribute to the chromatin landscape surrounding a DSB may also impose gene silencing effects. Indeed, removing this histone mark by ectopic expression of an H2A (K119/120R) mutant alleviated the DISC effect. The authors then went further to dissect the genetic bases for the DISC response. Based on previous work that identified the E3 ubiquitin ligases RNF8 and RNF168 in promoting uH2A foci formation at DSBs (Huen and Chen, 2010), Greenberg and colleagues found that co-depletion of the two ubiquitin ligases restored reporter activities. Conversely, silencing deubiquitinase USP16, which resulted in sustained DSB-associated uH2A signal, prolonged the DISC effect. Together, these three lines of evidence strongly implicate a functional coupling between DNA damage signaling, H2A ubiquitylation, and local transcriptional regulation.

Perhaps the most intriguing part of the study came from the possible functional distinction between DSB-associated mono-ubiquitylated H2A and K63-linked polyubiquitin chains that are known to be involved in DNA damage responses. Whereas accumulation of both classes of ubiquitin conjugates at DSBs depend on RNF8 and RNF168, the close correlation of uH2A and transcriptional silencing kinetics prompted the authors to link uH2A with DISC, while attributing K63-linked ubiquitin-modified species the primary, and perhaps the exclusive role in recruiting checkpoint and repair proteins, including BRCA1 and 53BP1, to DSBs. Although these data supports the interesting idea that multiple ubiquitin species co-exist at DSBs, with each devoted to a specific task, it remains enigmatic why reverting the DISC response requires simultaneous inactivation of both RNF8 and RNF168. Do these enzymes share similar substrates at the damage-modified chromatin? Do they independently target H2A for mono-ubiquitylation?

In addition, while this study unveils a novel role of ATM in regulating nascent transcriptional activities flanking a DSB, it remains largely speculative how its kinase activity promotes DISC. Given the requirement for uH2A in gene silencing, it is likely that ATM promotes DISC at multiple levels along the DNA damage-signaling cascade (**Figure 1**); by amplifying the γH2AX signal and/or by promoting DSB-association of uH2A ubiquitinases (i.e. RNF8 and RNF168). This model would then predict a requirement for MRN complex and MDC1 in promoting DISC, since these proteins are critical for ATM activation and focus accumulation of RNF8/RNF168 respectively.

Moreover, the current dogma holds that the chromatin becomes more accessible to DNA repair machineries shortly after DNA damage. In fact, ATM signaling seems to take a major part in ensuring proper repair of DSBs associated with heterochromatin structures as well as promoting global chromatin relaxation following DNA damage (Goodarzi et al., 2008; Noon et al., 2010; Ziv et al., 2006). In addition, signals arising from DSBs have also been reported to attract a number of chromatin remodeling complexes (Misteli and Soutoglou, 2009; Morrison and Shen, 2009). So, how does each of these activities integrate and complement each other to enforce optimal DNA repair and cell survival? How does the chromatin accommodate DISC while allowing access to DNA damage repair factors? Answering these questions will likely need a combination of biological tools, but will definitely reveal mechanistic insight into the interplay between DNA damage repair and chromatin biology.

Finally, what is the biological relevance of DISC? Coupled with the fact that ATM also plays a role in DNA replication checkpoints (Bartek et al., 2004), perhaps this ATM-dependent strategy in inhibiting transcription at the vicinity of DSBs is evolved to prevent collisions of DNA repair, DNA replication and transcription machineries. As we continue to thrive in understanding how cells respond and cope with genotoxic stress, ATM and its never-ending repertoire of DNA damage responses will continue to be under the spotlight in the decades to come.

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Figure Legend

Figure 1. ATM signaling mediates DNA Double-Strand Break Induced Silencing in Cis (DISC).

A model depicting how ATM regulates gene silencing and the components involved in DISC. At an actively transcribed region (step 1), a DNA double-strand break (DSB) is introduced, causing ATM activation and phosphorylation of the histone variant H2AX (step 2). ATM promotes assembly of the H2A ubiquitinases RNF8 and RNF168 to the vicinity of the DSB, which triggers H2A ubiquitylation, leading to inhibition of transcription (step 3). Upon repair of DSB or recovery from DNA damage signaling, USP16 deubiquitinases uH2A, resulting in restored transcription (step 4). Red and yellow circles represent H2AX and H2A molecules.