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DNA DAMAGE RESPONSE

The spliceosome cashes in at the ATM

> transcription-blocking … can activate non-canonical ATM signalling and modulate pre-mRNA splicing

The kinase ataxia-telangiectasia mutated (ATM) is a major coordinator of the DNA damage response (DDR). One consequence of DDR activation is the modulation of alternative splicing by mechanisms that were unclear. Tresini *et al.* now show that transcription-blocking DNA lesions induce the displacement of active spliceosomes, which can activate non-canonical ATM signalling and modulate pre-mRNA splicing.

The authors measured changes in chromatin composition following ultraviolet (UV) irradiation in quiescent human cells and found depletion in levels of the U2, U5 and U6 small nuclear RNAs and of the U2 and U5 small nuclear ribonucleoprotein splicing factors, which constitute the core of active spliceosomes. The depletion of these proteins from chromatin, which was caused by their displacement from DNA lesions, was prevented by inhibiting transcription. UV irradiation also decreased the interaction of splicing factors with elongating RNA polymerase II (Pol II). These results indicate that the displaced factors were actively involved in co-transcriptional splicing.

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As many core splicing factors are substrates of DDR kinases, the authors set out to identify which kinase is responsible for UV irradiation-dependent spliceosome displacement. Using various kinase inhibitors, they identified ATM as the kinase responsible. Examination of intron retention (which is an indicator of splicing efficiency) in several DDR and cell cycle genes showed that UV irradiation increased ATM-dependent intron retention and splicing impairment. Furthermore, RNA sequencing showed an ATM- and UV irradiation-dependent genome-wide increase in alternative splicing.

In search of the mechanism of ATM activation by transcription-blocking lesions, the authors hypothesized that spliceosome displacement would result in the formation of RNA–DNA hybrids (R-loops) between the RNA and single-stranded DNA adjacent to Pol II. R-loops could then activate ATM in the absence of canonical ATM activation signals. The R-loop-resolving factor RNase H1 was rapidly recruited to UV-irradiated sites in a transcription-dependent manner, and knocking down RNase H1 and RNase H2A activated ATM and promoted UV irradiation-mediated R-loop formation, spliceosome displacement and intron retention. Conversely, RNase H1 overexpression decreased UV-induced spliceosome displacement. Finally, combining treatments that either block transcription or promote ATM activation had an additive effect on spliceosome displacement and intron retention, indicating that ATM amplifies a pre-existing displacement signal initiated by transcription inhibition.

The results reveal the existence of a non-canonical ATM signalling pathway, which is activated in response to the impairment of co-transcriptional splicing. Following Pol II arrest at DNA lesions, spliceosome factors are displaced and R-loops are formed. This mediates ATM activation and leads to further spliceosome displacement and to a genome-wide increase in alternative splicing. Spliceosome displacement could promote repair by facilitating Pol II backtracking or removal at DNA lesions.

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ORIGINAL RESEARCH PAPER Tresini, M. *et al.* The core spliceosome as target and effector of non-canonical ATM signalling. *Nature* http://dx.doi.org/10.1038/nature14512 (2015)
FURTHER READING Shiloh, Y. & Ziv, Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nat. Rev. Mol. Cell Biol.* **14**, 197–210 (2013)

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