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DNA DAMAGE RESPONSE

Change of guard at the checkpoint

> "...is the ATM-to-ATR switch caused by DSB resection?"

Double-stranded DNA breaks (DSBs) activate two major DNA-damage checkpoint kinases, ataxia telangiectasia (A-T) mutated (ATM) and A-T and RAD3-related (ATR), which orchestrate the DNA damage response to delay cell cycle progression and allow the repair of DNA damage. However, the structural determinants of DNA that activate ATM and ATR and how the kinase activities are coordinated are poorly understood. Reporting in *Molecular Cell*, Shiotani and Zou provide new mechanistic insights.

Using human cell extracts and DNA molecules with defined structures to assay ATM activation *in vitro*, the authors found that long double-stranded DNA (dsDNA) with blunt ends induces ATM phosphorylation (a marker of activation) more efficiently than short dsDNA with blunt ends. By contrast, dsDNA with long single-stranded overhangs (SSOs) exhibits reduced ability to activate ATM, whereas dsDNA with short SSOs activates ATM efficiently. So, the activation of ATM by DSBs is regulated by the length of both dsDNA and SSOs.

DNA fragments with SSOs contain two types of ends: those of the dsDNA region (the junctions between the dsDNA and the ssDNA) and the ends of the SSOs. By chemically blocking these different dsDNA ends, Shiotani and Zou found that only the dsDNA–ssDNA junctions are crucial for ATM activation. Notably, the dsDNA–ssDNA junctions are also known to activate ATR. So, how are the activities of ATM and ATR coordinated at DSBs?

Exonucleases that resect linear DNA and progressively generate SSOs interfere with ATM activation but promote ATR activation. So, following the activation of ATM and the initiation of resection, SSOs might promote a switch between ATM and ATR at the DSBs. Indeed, checkpoint kinase 2 (CHK2), an ATM substrate, is transiently phosphorylated shortly after cells are damaged by ionizing radiation (IR) and the decline in CHK2 phosphorylation coincides with the activation of CHK1, an ATR substrate. Notably, replication protein A (RPA)-coated ssDNA foci (which are generated by the exonuclease-mediated resection of DSBs and are known to recruit ATR) accumulate at DSBs when the activation of ATM is attenuated. So, is the ATM-to-ATR switch caused by DSB resection?

This seems to be the case, as ATM induces DNA resection and an ATM inhibitor blocks the ATM-to-ATR switch following exposure to IR. Furthermore, expression of exonucleases that are involved in resection promotes the IR-induced switch from ATM to ATR at DSBs. So, the authors propose that "the ATM-to-ATR switch driven by DSB resection is the key mechanism through which the functions of ATM and ATR are coordinated and integrated".

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ORIGINAL RESEARCH PAPER Shiotani, B. & Zou, L. Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Mol. Cell* **13**, 547–558 (2009)

FURTHER READING Cimprich, K. A. & Cortez, D. ATR: an essential regulator of genome integrity. *Nature Rev. Mol. Cell Biol.* **9**, 616–627 (2008)