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DNA REPLICATION

Looping smoothens repetitive DNA replication

> the accumulation of DNA supercoiling and the formation of DNA loops, is important for the suppression of the ATR checkpoint

Genomes contain extensive regions of repetitive sequences that can potentially impair DNA replication, owing to their propensity to adopt unusual conformations. The mechanisms underlying the replication of such regions are poorly understood. In *Nature Cell Biology*, Costanzo and colleagues report that repetitive centromeric DNA forms loops that repress the Ser/Thr protein kinase ATR-dependent checkpoint, which monitors replication fork progression, thus facilitating their replication.

To study the dynamics of repetitive DNA replication, the authors used *Xenopus laevis* egg extracts and bacterial artificial chromosomes (BACs) containing either human centromeric alpha-satellite DNA (which is made of 171 base pair-long repeat arrays) or non-centromeric repeat-free DNA regions. Replication efficiency, the binding of replication proteins and replication initiation were similar in these BACs, but replication kinetics were slower in the BAC-containing centromeric DNA. Moreover, when the authors set out to identify proteins enriched or depleted in centromeric DNA during replication using quantitative mass spectrometry, they revealed differences between proteins associated with repetitive and repeat-free chromatin. Factors that were enriched in centromeric BACs included DNA repair proteins such as mismatch repair (MMR) proteins, and structural proteins such as condensins, which are protein complexes that encircle DNA and have several regulatory functions and especially contribute to mitotic chromosome condensation, albeit through a poorly understood mechanism.

Together, slower replication and repair factor enrichment suggested the presence of abnormal structures in centromeric DNA, which may affect replication fork progression. To assess this possibility, the authors analysed ATR checkpoint signalling and found that treatment with a DNA polymerase inhibitor, which induces replication fork stalling and stress, unexpectedly did not activate ATR checkpoint signalling at centromeric DNA. Moreover, accumulation of replication protein A (RPA) on single stranded DNA (ssDNA), which is crucial for ATR activation, was reduced.

When performing electron microscopy on centromeric DNA, the authors observed large regions of ssDNA or 'bubbles', which they hypothesized to be caused by DNA supercoiling. Indeed, positively supercoiled DNA accumulated at centromeric DNA, and this was dependent on the function of topoisomerase I and, possibly, condensins. Moreover, the authors observed double-stranded DNA loops at centromeric chromatin, which are probably also a result of supercoiling, which were held together by a condensin-rich protein matrix (the exact nature of the matrix remains to be determined). Importantly, they found that DNA supercoiling inhibited RPA binding to chromatin and thus prevented ATR checkpoint activation. Notably, centromeric DNA replication was prevented if the ATR checkpoint was induced.

Thus, centromeric DNA topology, which involves the accumulation of DNA supercoiling and the formation of DNA loops, is important for the suppression of the ATR checkpoint and for proper replication at centromeric DNA. Whether the same mechanisms operate at other repetitive regions such as telomeres remains to be determined.

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ORIGINAL ARTICLE Aze, A. *et al.* Centromeric DNA replication reconstitution reveals DNA loops and ATR checkpoint suppression. *Nat. Cell Biol.* http://dx.doi.org/10.1038/ncb3344 (2016)

FURTHER READING Cimprich, K. A. & Cortez, D. ATR: an essential regulator of genome integrity. *Nat. Rev. Mol. Cell Biol.* **9**, 616–627 (2008) | McKinley, K. L. & Cheeseman, I. M. The molecular basis for centromere identity and function. *Nat. Rev. Mol. Cell Biol.* **17**, 16–29 (2016)