How to make a replication origin in multicellular eukaryotes

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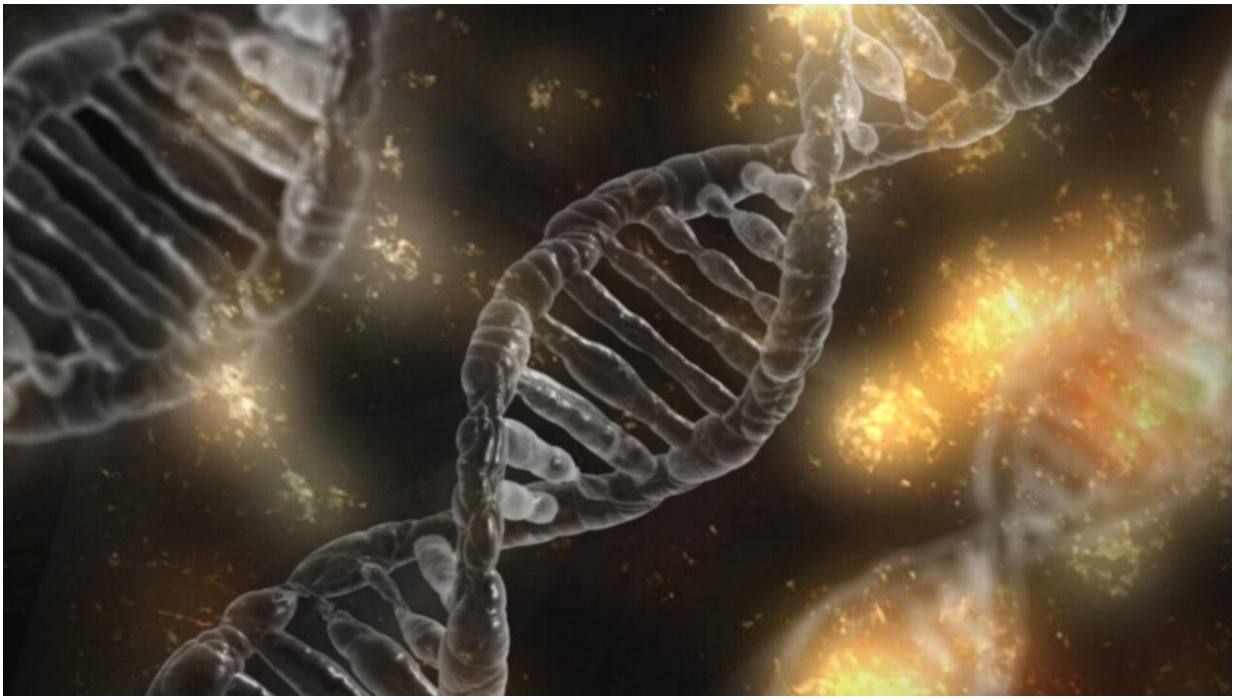

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Loading of replicative helicases onto DNA is a key event during the initiation of chromosomal DNA replication. It takes place at specific chromosomal regions termed origins and is facilitated by the ORC protein complex. By resolving the cryo-EM structures of DNA-bound ORC, researchers from the Bleichert group (now at Yale) broaden our understanding of how DNA replication is initiated in animals.

Accurate replication of chromosomal DNA is essential for the survival and propagation of living organisms. Prior to cell division, many different proteins work together and duplicate genomes by semi-conservative replication so that copied chromosomes can be segregated into daughter cells. Genome integrity is sustained by highly efficient and accurate DNA replication exactly once per cell cycle. Failure to replicate DNA precisely can alter gene copy number and chromosome ploidy, which can give rise to genomic instability and a variety of human diseases.

In higher eukaryotes, DNA replication is initiated at thousands of genomic sites termed replication origins. A multi-subunit protein complex, the Origin Recognition Complex (ORC), binds these origins and is essential for replication onset as it loads the replicative helicase onto DNA. In yeast, origins are defined by a conserved consensus sequence that is recognized by ORC. By contrast, how replication origins are defined in animals (or metazoans) has remained unclear, but chromatin cues and local DNA structure are thought to help mediate the recognition of the origins.

Jan Marten Schmidt dedicated his Ph.D., which he did in the group of Franziska Bleichert (who moved from the FMI to Yale in early 2020), to answering the long-asked question of how metazoan ORC engages origin DNA without the guidance of a consensus sequence. He wanted to know how DNA geometry, rather than a specific sequence, may influence ORC binding and ultimately helicase loading to better understand how origins are defined in metazoans.

Schmidt and Bleichert solved several structures of DNA-bound Drosophila ORC with cryo-electron microscopy. The structures revealed that the ATPase domains of metazoan ORC have evolved multiple elements that make contacts with DNA, encircling the DNA to stabilize the initiator on origins, and bending DNA. Furthermore, the researchers

showed that ORC preferentially binds to AT-rich DNA, that DNA binding by ORC can be uncoupled from DNA bending, and that DNA bending promotes helicase loading in vitro.

Schmidt summarizes the main findings and relevance of the study: "We suggest that DNA geometry is important for ORC-mediated helicase loading and may help determine the location of metazoan origins in vivo. In addition, we believe that our in vitro metazoan helicase loading assay is a useful tool to test the effect of different DNA substrates, proteins and ORC mutants on helicase loading in the future. Our findings will help the research community to further address how origins are selected for replication in humans."

More information: Jan Marten Schmidt et al. Structural mechanism for replication origin binding and remodeling by a metazoan origin recognition complex and its co-loader Cdc6, *Nature Communications* (2020). DOI: 10.1038/s41467-020-18067-7

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