DNA REPLICATION

Shaping up for a new start

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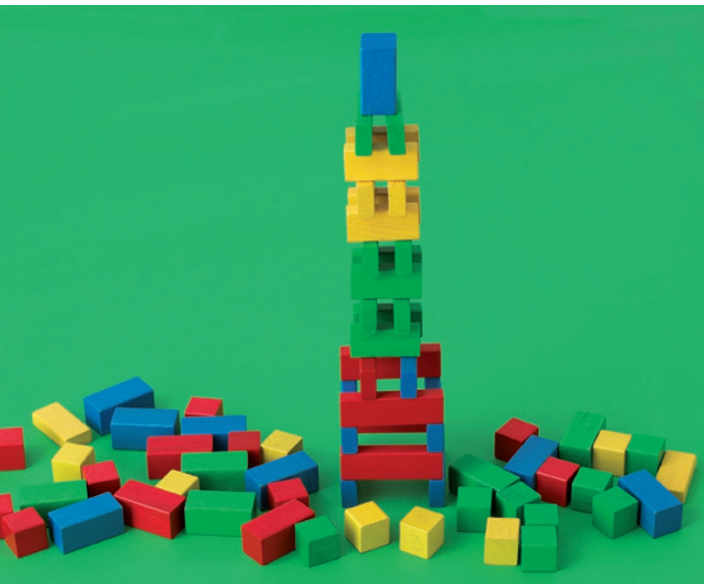
The initiation of DNA replication is mechanistically similar in archaea and eukaryotes. Initiator proteins, which typically comprise winged helix domains (WHDs) and an ATPase module that belongs to the AAA$^+$ superfamily, are essential to replication initiation and facilitate the formation of higher-order assemblies of replication proteins on replication origins. Two new studies now show the crystal structures of three archaeal initiator proteins bound to replication origin DNA, providing new insights into how initiators recognize and reshape origins to initiate DNA replication.



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Using multiwavelength anomalous dispersion, James Berger and colleagues solved the structure of the co-crystallized initiator proteins Orc1-1 and Orc1-3 from *Sulfolobus solfataricus* on a 33-bp fragment of DNA encompassing their binding sites. Orc1-1 and Orc1-3 form a large, positively charged surface that requires WHD–DNA and, surprisingly, AAA$^+$–DNA contacts. Both the helix-turn-helix (HTH) and β-hairpin wing motifs that comprise the WHD bind over a full turn of DNA, which forms a larger WHD–DNA interface than conventional modes. Furthermore, mutations in the HTH or β-hairpin wing motifs reduced the affinities of Orc1-1 and Orc1-3 for their binding sites, and binding was completely abolished by simultaneous mutation of the HTH and β-hairpin wing motifs.

In a separate study, Dale Wigley and colleagues determined the crystal structure of Orc1, an initiator protein in *Aeropyrum pernix*, bound to a 22-bp fragment of DNA containing its specific target site, *ori1*. The WHD of Orc1 bound DNA in a canonical manner and led to widening of the helix, which further facilitated the Orc1–DNA contacts. They also showed that the AAA$^+$ domain of Orc1 made substantial contact with the DNA; the contacts observed in the crystal structures were consistent with DNA footprint experiments. Moreover, using isothermal calorimetry, they showed that a single WHD bound a single origin recognition box (ORB; two pairs of which surround the replication start site within *ori1*).

Both groups showed that the initiator proteins made few sequence-specific contacts with the DNA; thus, binding specificity was mostly determined by contacts with the backbone of the double helix. In addition, the binding of Orc1-1 and Orc1-3 to origin DNA resulted in substantial unwinding of the DNA double helix and introduction of a 20° bend. Orc1 binding in *A. pernix* introduced a 35° bend and helical unwinding so that the twist of the helix was reduced to 11 bp per turn. Crucially, the initiator proteins bound to DNA asymmetrically so that DNA unwinding was directed towards the replication start site, which could also facilitate higher-order assembly of replication proteins.

Many more questions remain about the mechanism by which DNA replication is initiated, including whether this mechanism is conserved through evolution.

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ORIGINAL RESEARCH PAPERS
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