

Structure of Origin Recognition Complex Bound to Autonomously Replicating Sequence

Ning Gao

State Key Laboratory of Membrane Biology, Peking-Tsinghua Center for Life Sciences, School of Life Sciences, Peking University, Beijing 100871, China

gaon@pku.edu.cn

The Origin Recognition Complex (ORC) consists of six highly conserved subunits, Orc1-6. Despite the conservation in protein sequence, ORC from different species have divergent DNA binding specificities ranging from an absolute requirement for a specific sequence in *Saccharomyces cerevisiae* to no known sequence requirement in humans. Yet ORC of all species bind DNA to mark the site for DNA replication initiation. Here, we report a 3-Å cryo-EM structure of *S. cerevisiae* ORC bound to a 72-bp origin DNA containing the ARS consensus sequence (ACS) and B1 element. ORC encircles DNA through extensive interactions with both phosphate backbone and bases, and bends DNA at the ACS and B1 sites. A key structural feature is that a conserved basic amino acid patch of Orc1 inserts deeply into the minor groove of the ACS for specific base recognition. In addition, a species-specific helical insertion located in the winged-helix domain of Orc4 is embedded in the major groove of the ACS through hydrophobic interactions with a stretch of conserved thymines. Importantly, these base-specific contacts at the ACS are mostly with invariant bases. Moreover, similar major and minor-groove insertions are also embedded in the B1 site by basic patch motifs from Orc2 and Orc5, respectively, to contact bases and to bend DNA. Our findings show that yeast ORC binds thymine-rich DNA in a universally conserved mechanism mainly via the Orc1 basic patch element but acquires its species-specific sequence recognition from the divergent Orc4 insertion helix. This work pinpoints a conserved role of ORC in modulating DNA structure to facilitate origin selection and helicase loading in all eukaryotes.