

Cryo-EM Structure of the Human Pre-replication Complex

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The minichromosome maintenance (MCM) 2-7 double hexamer (DH) contains two heterohexameric rings joined at the N-terminal ends. It is loaded onto duplex DNA by the origin recognition complex and serves as the scaffold for the assembly of the bidirectional replisomes as well as the catalytic core of the CMG (Cdc45-MCM2-7-GINS) helicase. Replisome assembly is accompanied by MCM-DH remodeling and uncoupling that respectively melts and separates the origin DNA strands. To better understand the role of MCM2-7 in this melting process, we recently determined a 2.59-Å cryo-electron microscopy structure of the human MCM-DH (hMCM-DH), also known as the pre-replication complex (pre-RC). The overall structure of the hMCM-DH is similar to that of the yeast MCM-DH but shows a more constricted central channel with a diameter of only 13 Å at the hexamer interface. This unusual conformation untwists and stretches the DNA strands such that almost a half turn of the bound duplex DNA is distorted with 1 base pair completely separated, generating an initial open structure (IOS) at the hexamer junction. Disturbing the IOS inhibits DH formation and replication initiation. Mapping of hMCM-DH footprints indicates that IOSs are distributed across the genome in large clusters aligning well with initiation zones designed for stochastic origin firing. This work unravels an intrinsic mechanism that couples DH formation with initial DNA melting to license replication initiation in human cells. Our findings also highlight that although general mechanisms obtained from yeast could be applied to human cells, the detailed regulation of pre-RC assembly is drastically different between yeast and human.