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The architecture and function of the chromatin replication machinery

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Genomic DNA in eukaryotic cells is packaged into nucleosome arrays. During replication, nucleosomes need to be dismantled ahead of the advancing replication fork and reassembled on duplicated DNA. The architecture and function of the core replisome machinery is now beginning to be elucidated, with recent insights shaping our view on DNA replication processes. Simultaneously, breakthroughs in our mechanistic understanding of epigenetic inheritance allow us to build new models of how histone chaperones integrate with the replisome to reshuffle nucleosomes. The emerging picture indicates that the core eukaryotic DNA replication machinery has evolved elements that handle nucleosomes to facilitate chromatin duplication.

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Introduction: duplicating eukaryotic chromosomes

Eukaryotic chromosome replication involves both the duplication of DNA and of the complex epigenetic environment that protects and regulates the genome. Replication therefore entails three distinct tasks: *i.* disrupting the nucleosomes that form chromatin ahead of the replicative machinery, *ii.* coupling DNA unwinding and synthesis and *iii.* repopulating chromatin on duplicated DNA [1]. To perform these functions, the eukaryotic replisome has evolved numerous ways to interact with histones through core or accessory components [2]. Here, we highlight recent discoveries that improve our understanding of DNA replication mechanics and discuss unique features of the eukaryotic replisome that allow the duplication of chromatin and not just DNA.

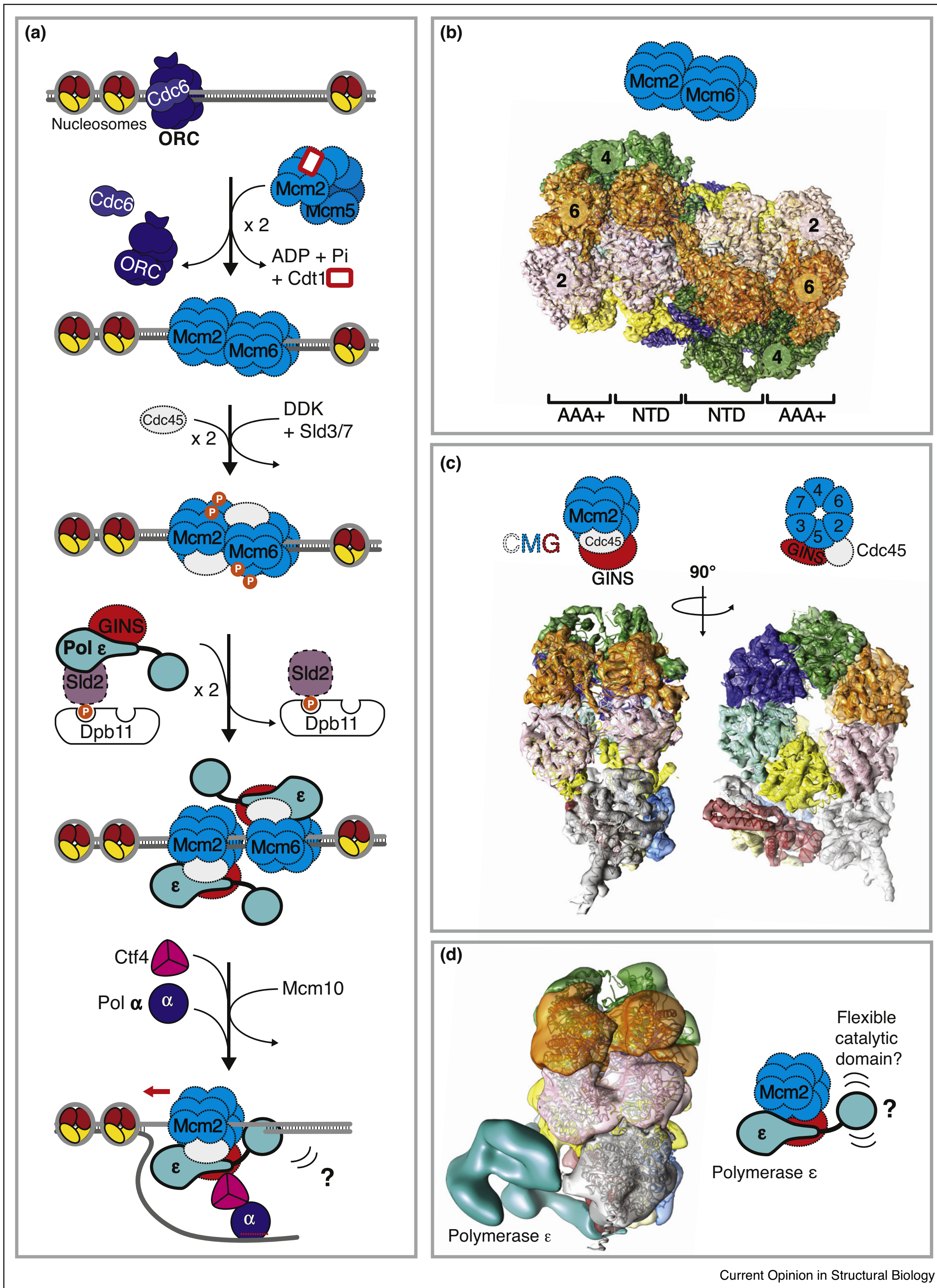
The chromatin landscape defines eukaryotic origins of replication

DNA replication start sites are recognized by the Origin Recognition Complex (ORC), which accesses sites on the genome that are naturally nucleosome free [3]. While in prokaryotes the recognition of specific DNA sequences is a major mechanism of origin selection, this is not true for most eukaryotes. One exception is *Saccharomyces cerevisiae*, which contains recognizable autonomous replicating sequences [2]; however, even in budding yeast, sequence recognition is not the only determinant for origin discrimination with the chromatin landscape also playing an important role. Notably, in DNA replication reactions reconstituted *in vitro*, initiation is sequence-specific on chromatinised but not naked DNA [4••,5•]. In this context, chromatin likely impairs non-specific DNA-association and directs ORC to origin sites. Nucleosomes flanking the replication start sites also have an important role in ORC recruitment, for example through a dimethylated-histone H4 interaction with a bromo-adjecent homology domain found in the ORC subunit, Orc1 (Figure 1**a**) [6]. In summary, nucleosomes appear to play a dual role imparting origin selectivity by *i.* physically preventing non-specific ORC association with DNA and *ii.* serving as a docking site for ORC deposition onto origins.

Mechanisms of origin activation

Why are stretches of nucleosome-free DNA needed to initiate replication? Origin firing requires the assembly of two symmetric replisome complexes, which, once activated, move bidirectionally to open a replication bubble (Figure 1**a**). The ORC ATPase complex [7], together with Cdc6, mediates the loading of two copies of the hexameric, ring-shaped MCM helicase (a AAA+ ATPase recruited in complex with Cdt1) onto origin DNA [8]. While the molecular mechanism of MCM recruitment, and the number of ORC assemblies required for this process are debated [2,9], it is clear that the ATPase function of MCM is instrumental in releasing Cdt1 and completing helicase loading (Figure 1**a**) [10,11]. The result of the loading reaction is the formation of an inactive head-to-head double hexamer that encircles, and might locally destabilize, duplex DNA (Figure 1**b**) [12–14]. Subsequent MCM helicase activation depends on the association of two factors named Cdc45 and GINS (forming the CMG holo-helicase) [8], whose recruitment is controlled by two kinases, DDK and CDK. DDK phosphorylates the N-terminal tails of Mcm4/Mcm6, probably facilitating the symmetric deposition of two copies of Cdc45 onto each MCM, mediated by a Sld3/Sld7 dimer of

Figure 1



Activation of a eukaryotic origin of replication. **(a)** ORC recognizes a nucleosome free origin of replication. With Cdc6, ORC mediates the loading of the Mcm-Cdt1 heptamer. Upon ATP hydrolysis by MCM, Cdt1 is released and a head-to-head MCM double hexamer is origin-loaded. Cdc45 becomes MCM-associated via Sld3/7 after DDK phosphorylation of MCM. GINS/Pol epsilon are recruited in the context of a pre-loading complex. Origin activation depends on Mcm10 and Pol alpha. **(b)** Cryo-EM structure of the yeast MCM double hexamer (EMD-6338). **(c)** Cryo-EM structure of the *Drosophila* CMG helicase (EMD-3318). **(d)** EM structure of the CMG-Pol epsilon complex (EMD-64650).

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