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Mechanisms for Initiating Cellular DNA Replication
Costa, Hood, and Berger

Supplemental Material

Control of origin accessibility

Bacteria. In *E. coli*, the ability of DnaA to access its binding sites within *oriC* is closely tied to the action of dedicated DNA-binding proteins that either compete for origin sites or stimulate DnaA association and action (**Figure 6a**). For example, in *E. coli*, IHF and HU are two NAPs that synergistically potentiate the action of DnaA on *oriC* (1). Both proteins significantly bend DNA (2-4), an activity that can either help destabilize duplex origin DNA directly (HU) (1, 5, 6), or promote the assembly of ATP-bound DnaA on *oriC* to drive DUE melting (IHF) (1, 3, 7-10). By contrast, a third NAP – Fis (Factor for Inversion Stimulation) – can prevent the binding of IHF to *oriC* and thereby block DnaA assembly (11-15). Although neither IHF nor Fis are essential for viability, both factors are involved in maintaining initiation synchrony during rapid *E. coli* growth (6, 16).

Origin accessibility is also regulated to prevent inappropriate re-initiation. For example, following initiation in *E. coli*, a dedicated protein known as SeqA binds to newly-replicated origins, enforcing synchronous DNA replication (17). Following the passage of a replication fork through *oriC*, the origin enters into a transient, hemi-methylated state that allows SeqA – whose affinity for hemi-methylated DNA is greater than for fully-methylated substrates (18, 19) – to engage specific GATC sequences within the region (20-22). As SeqA associates with *oriC*, it assembles into a filamentous oligomer, organizing the origin into a stable nucleoprotein complex in which SeqA occupies low-affinity DnaA sites to block initiator binding to *oriC* and impede re-replication (22-24). Upon dissociation of SeqA from DNA, the Dam methylase fully methylates *oriC* to prevent SeqA from rebinding the origin (25), thereby re-establishing initiation competency.

Other bacteria do not appear to rely on the NAPs and SeqA system used in *E. coli*. Nevertheless, the use of sequence-specific DNA binding proteins to compete for DnaA access to cognate replication origins is a general trend. For example, in *B. subtilis,* an origin-binding protein called SpoA both recognizes specific sites in *oriC* and prevents DNA melting *in vitro,*

possibly through an origin sequestration mechanism (26). In *Caulobacter crescentus,* a master regulatory protein known as CtrA silences the chromosomal origin (*Cori*) by binding next to one or more DnaA boxes, thereby preventing the initiator from appropriately engaging DNA (27-30). Notably, control of CtrA function utilizes several cell-cycle dependent strategies reminiscent of those found in eukaryotes (discussed below). For example, CtrA can be phosphorylated by the histidine kinase CckA, an event that allows CtrA to productively associate with C_{ori} (29). CtrA levels also are regulated by controlled proteolysis through the ClpXP degradasome (31, 32). Given the relative abundance of DNA-binding and remodeling proteins in bacterial cells (33), it seems likely that studies of other bacterial species will turn up analogous, albeit distinct, systems for controlling origin accessibility.

Eukaryotes. Early genome-wide analyses with yeast first revealed that the firing of replication origins follows a complex temporal and spatial pattern that does not perfectly overlap with predicted ACS loci (34) (35). More recent analyses have highlighted a key role for nucleosomes and their positioning in defining origin regions (36). Nucleosomes comprise an octameric histone core that wraps duplex DNA into a solenoidal supercoil (37). Histones and their terminal tails are modified by an array of covalent marks, including acetylation and methylation(38), ubiquitinylation(39) and ADP-ribosylation(40). These modifications provide an extensive combinatorial code for fine-tuning nucleosome position, stability, compaction, and protein-chromatin interactions (41).

As with other DNA-dependent transactions (e.g., transcription and repair), initiation of DNA replication in eukaryotes is strongly influenced by chromatin status and environment. For example, conserved patterns of nucleosome positioning and occupancy are evident in many yeast origins (42, 43). *In vitro* reconstitution assays have shown that nucleosomes naturally tend to avoid binding within ACS sequences (44) and that the addition of ORC and ATP further can reorder nucleosomes to positions observed *in vivo* (43). The N-terminal BAH domain of Orc1, which is essential for activation of certain origin loci (45), may contribute to nucleosome organization around replication origins (46).

Interestingly, the chromatin flanking known ORC binding sites is dynamic and characterized by high nucleosome turnover (47). At present, it is unclear whether ORC helps to reposition nucleosomes directly or whether it acts indirectly by influencing the activity of chromatin remodeling enzymes (48). Within higher eukaryotes, nucleosome positioning and mobility around replication origins seem to follow some of the patterns seen in yeast. For example, ORC binding sites in *Drosophila* and in Chinese hamster frequently are devoid of nucleosomes and reside next to dynamic chromatin regions (49, 50).

As with transcription, DNA replication requires DNA opening events that are guided by specific chromatin cues (reviewed in (36)). Consistent with this theme, the genome-wide distribution of site-specific transcription factors in *Drosophila* (such as Myb, also refer to Section 2.2) overlaps with ORC binding sites (51, 52). Metazoan replication origins generally do not reside within intergenic regions (51) (although they can in yeast (34)), suggesting that these replication start sites, which overlap with actively transcribed regions, may be activated more readily than other DNA segments. Indeed, promoter sequences themselves may directly help define or lead to origin activation, possibly by ensuring that DNA is accessible to replication initiation factors (53). However, transcriptional activity also can also silence initiation sites, such as when an origin sits within the boundaries of an actively transcribed gene (54, 55).

Further insights into the interdependence between replication, transcription, and the chromatin landscape derive from developmental patterns of origin firing. For example, selectively replicated DNA regions in *Drosophila* polytene salivary gland chromosomes are transcriptionally silent and enriched in repressive histone modification signatures (56). Notably, ORC occupancy is markedly diminished within the same regions, indicating that under-replication is enforced by reducing productive initiation events (57). Altogether, it appears that eukaryotic replication start sites are defined by a ‘code’ that is distinct from DNA sequence, and instead depend on contextual cues that influence DNA packaging and topology. However, the mechanisms by which this code is manifest and read out still remain largely undefined.

Archaea. How archaea control the ability of initiation factors to associate with origin regions is similarly poorly understood. Nonetheless, the pervasiveness of origin-sequestration strategies

in bacteria and eukaryotes suggests that archaea will employ analogous approaches. Most archaeal phyla contain one or more NAPs that assist with DNA packaging, and hence could potentially influence replication onset. For example, euryarchaeota and certain crenarchaeota encode two homologs of eukaryotic core histones that assemble on DNA as a dimer of dimers (58); however, archaeal histones also lack N- and C-terminal tails (59), suggesting that post-translational modification events may play less of a role in controlling DNA-binding and remodeling than in eukaryotes. Crenarchaeal species that lack histones contain a highly-abundant protein, Cren7, which can constrain negative supercoils and modulate higher-order DNA organization (60). Many crenarchaeota (and some euryarchaeota) encode a factor, Alba (or Sso10b/Sac10b), which also binds and compacts DNA (61-64). Alba can both bind DNA and impede MCM helicase progression in a manner subject to post-translational control (particularly acetylation) (65-67), an activity directly linked to cell cycle progression (68). Additional studies are needed to build upon these early efforts for clarifying whether and how archaeal NAPs and other DNA-binding proteins play a role in governing the timing and location origin firing.

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