

Getting a Grip on Licensing: Mechanism of Stable Mcm2-7 Loading onto Replication Origins

A recent *Molecular Cell* paper by [Randell et al. \(2006\)](#) sheds light on the role of ATP hydrolysis by Cdc6 in promoting the stable loading of the Mcm2-7 complex onto origins of DNA replication.

Eukaryotic cells duplicate their vast genomes rapidly by initiating DNA replication from hundreds or even thousands of origins during S phase. The process of replication initiation is divided into two steps, which ensures that each origin initiates DNA replication only once during S phase. During the first step, which occurs in G1, a prereplication complex (pre-RC) is assembled at the origin. Pre-RC assembly involves the sequential binding of the origin recognition complex (ORC), Cdc6, Cdt1, and Mcm2-7 to the origin (see [Figure 1](#), reprinted from [Randell et al. \[2006\]](#)). The Mcm2-7 complex likely functions as the replicative DNA helicase, but it is initially recruited to pre-RCs in an inactive form. Interestingly, it appears that ~20 Mcm2-7 complexes are recruited to each origin of DNA replication in each G1 phase ([Bowers et al., 2004](#); [Edwards et al., 2002](#)). During the second step of replication initiation, which takes place as cells enter S phase, two Mcm2-7 complexes are thought to be activated as DNA helicases at each origin, DNA is unwound bidirectionally, and the replisome can be assembled. More than one initiation event from the same origin does not occur because origin firing triggers loss of Mcm2-7 complexes from the origin, and rebinding of Mcm2-7 to chromatin is blocked until cells exit mitosis ([Diffley, 2004](#)). A remarkable feature of the above model is that all origins assemble pre-RCs in G1, yet some origins do not undergo initiation until late in S phase, which can occur many hours later. It follows that the Mcm2-7 complex must form an extremely stable interaction with the DNA.

Although the requirement for ORC, Cdc6, and Cdt1 in recruiting Mcm2-7 to origins has been established for some time, the mechanism has remained uncharacterized. One hint comes from the fact that many of the proteins involved in pre-RC formation are ATPases. New results from the Gautier lab indicate that the ATPase activity of the Mcm2-7 complex is not essential for its recruitment to origins ([Ying and Gautier, 2005](#)). In a recent issue of *Molecular Cell*, Bell and colleagues have investigated what role ATP hydrolysis by Cdc6 plays in pre-RC formation and how this event is coordinated with ATP hydrolysis by ORC ([Randell et al., 2006](#)). The authors demonstrate that Cdc6 hydrolyzes ATP only in the presence of ORC and origin DNA, presumably after Cdc6 binds the ORC-DNA complex. Interestingly, a Cdc6 mutant that is unable to hydrolyze ATP can recruit Mcm2-7 to the origin, but the association is weak, being

salt sensitive, and the number of Mcm2-7 complexes loaded per origin is reduced. As shown previously ([Bowers et al., 2004](#)), an ATPase-deficient ORC mutant also results in reduced loading of Mcm2-7, but the Mcm2-7 complexes that are recruited interact tightly with DNA, being salt resistant. When both mutants are present, the Cdc6 phenotype predominates, suggesting that the Cdc6 ATPase step functionally precedes the ORC ATPase step. Together, the results suggest that the complex of ORC and Cdc6 loads individual Mcm2-7 hexamers in a cycle consisting of three steps (see [Figure 1](#)). First, Mcm2-7 is recruited to the origin by ORC and Cdc6 independently of ATP hydrolysis. Second, ATP hydrolysis by Cdc6 stimulates the stable association (or “loading”) of Mcm2-7 with origin DNA. Finally, ATP hydrolysis by ORC allows the cycle to begin again, resulting in the loading of multiple Mcm2-7 complexes per origin.

The authors also showed that Cdt1 is required for Mcm2-7 loading in their cell-free system. Intriguingly, Cdt1 could be detected on the origin only when Cdc6 ATP hydrolysis was disrupted. The authors speculate that Mcm2-7 enters the origin in a complex with Cdt1 and that Cdc6 ATP hydrolysis stimulates dissociation of Cdt1. Cdt1 dissociation may in turn trigger the stable interaction of Mcm2-7 with the DNA.

The results generate a detailed mechanistic framework for pre-RC formation. The dependence of Cdc6 ATP hydrolysis on ORC, and the sequential action of the Cdc6 and ORC ATP hydrolysis steps, has important implications for the regulation of pre-RC formation. First, Cdc6 can only act on the Mcm2-7 complex in the context of origins of DNA replication. This would prevent futile dissociation of Cdt1 from Mcm2-7. Second, ORC cannot initiate pre-RC formation in the absence of Cdc6.

The results in this paper raise new questions while re-emphasizing old issues. First, although Cdc6 ATP hydrolysis is ORC dependent, it does not appear to be Mcm2-7-dependent, raising the possibility of futile cycles of Cdc6 hydrolysis. To be sure, the rate of Cdc6 hydrolysis appears to be slow, and it will be interesting (though difficult) to test whether it is stimulated by the arrival of Mcm2-7 at the origin. Second, what is the nature of the interaction between the Mcm2-7 complex and DNA before and after Cdc6 ATP hydrolysis, and are these intermediates physiological? For example, is Mcm2-7 topologically engaged with the DNA after the Cdc6 hydrolysis step, and, if so, does it encircle double-stranded or single-stranded DNA? This distinction has profound implications for the mechanism of downstream events of helicase activation and origin unwinding ([Takahashi et al., 2005](#)).

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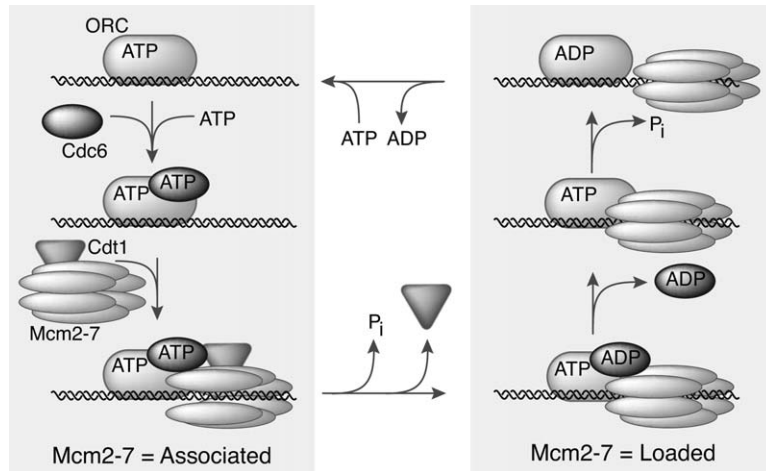


Figure 1. A Model for Mcm2-7 Loading by the ORC and Cdc6 ATPases

ATP bound ORC first binds origin DNA. Cdc6 then binds ORC and ATP. Cdt1 and Mcm2-7 (possibly as a complex) associate with ORC and Cdc6 at the origin. ATP hydrolysis by Cdc6 leads to the loading of Mcm2-7 complexes on DNA and the release of Cdt1 from the origin. Cdc6 association is destabilized by Cdc6 ATP hydrolysis. ATP hydrolysis by ORC completes the Mcm2-7 loading reaction allowing further rounds of Mcm2-7 loading. The figure and figure legend are reprinted from [Randell et al. \(2006\)](#).

Selected Reading

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Modified View of tRNA: Stability amid Sequence Diversity

In a recent issue of *Molecular Cell*, a report from the Phizicky lab challenges two commonly held notions regarding tRNAs: (1) tRNAs are stable molecules shielded from the machinery that cause other RNAs to have short half-lives, and (2) the many modifications found on tRNAs serve only subtle biological functions. [Alexandrov et al. \(2006\)](#) show that tRNA modifications can play an important role in tRNA stability, as without particular modifications, tRNA half-life can be reduced from hours to minutes by a new turnover pathway.

RNA molecules from all forms of life have been found to contain chemical modifications of nucleosides that are added to the RNA posttranscriptionally—over a hundred different modifications in all ([Grosjean and Benne, 1998](#), [Grosjean, 2005](#)). These modifications serve a variety of functions, often providing specialized recognition for use in cellular pathways. Eighty percent of these modifications are found in the transfer RNA (tRNA) molecules that bring specific amino acids into the peptidyl transferase centers of ribosomes, thus decoding the genetic code expressed in messenger RNAs.

A question that has long intrigued RNA researchers is why so many modifications of the tRNAs are retained. Genetic studies in bacteria and yeast have shown that

the great majority of the tRNA modification enzymes are not essential for life and, indeed, that deletion of individual modification enzymes have no serious growth phenotypes. Although a small number of modifications near the termini and anticodons of tRNAs have been shown to affect aminoacyl charging and fine tuning of codon recognition ([Hopper and Phizicky, 2003](#), [Agris, 2004](#)), many of the modifications occur at positions that would not obviously impact these functions. Two prominent explanations have been discussed for the frequency and diversity of tRNA modifications. The first is that the individual modifications provide additional options for highly selective molecular recognition of specific tRNAs. The second explanation is an answer to a unique structural problem faced by tRNA molecules. Transfer RNAs must all conform, within narrow parameters, to a specific tertiary structure to fit into the ribosomal active sites and also be recognized by common processing enzymes (e.g., RNase P). However, each tRNA must have unique sequence determinants to be recognized as the carrier of a different amino acid. In varying the sequences of the dozens to hundreds of different tRNAs needed by the cell, it is inevitable that certain sequences will be less likely to fold into the requisite structure or to maintain a single, stable structure once folded.

In yeast modification of tRNA at position G46 to 7-methyl-G (m^7G_{46}) is catalyzed by the collaboration of the yeast *TRM8* and *TRM82* gene products ([Alexandrov et al., 2005](#)). [Alexandrov et al. \(2006\)](#) show that cells lacking *TRM8* or *TRM82* and any one of seven tested other