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Putting Two Heads Together to Unwind DNA

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The loading of replicative helicases onto DNA is tightly regulated in all organisms, yet the molecular mechanisms for this event remain poorly defined. Remus et al. (2009) provide important insights into helicase loading in eukaryotes, showing that the Mcm2–7 replicative helicase encircles double-stranded DNA as head-to-head double hexamers.

The duplication of a genome requires unwinding of the DNA duplex to allow for replisome action. This event is primarily catalyzed by DNA helicases that separate the complementary DNA strands in an ATP-dependent process. In eukaryotes, an inactive form of the Mcm2–7 replicative helicase is loaded onto DNA during G1 phase to form the prereplicative complex (pre-RC), which marks all potential origins of replication. At the onset of S phase, the helicase is activated and the replisome is assembled. The temporal separation of helicase loading and activation ensures that the genome is replicated exactly once per cell cycle (Sclafani and Holzen, 2007). In this issue, Remus et al. (2009) provide compelling evidence that the Mcm2–7 helicase is initially loaded onto DNA as a head-to-head double hexamer with double-stranded DNA (dsDNA) running through a central channel (Figure 1B). This insight has important implications for the mechanisms of Mcm2–7 helicase activation and function.

The Mcm2–7 helicase is an elongated heterohexameric complex composed of one copy each of the six highly related Mcm2–7 proteins (Figure 1). Each Mcm subunit includes a C-terminal AAA+ ATPase domain and a distinct N-terminal domain. Studies of the archaeal homohexameric Mcm complex reveal a hexamer with two distinct lobes in which the AAA+ domains comprise the larger lobe and the N-terminal domains are found at the opposite end (Pape et al., 2003) (Figure 1A). In vitro studies of DNA unwinding by Mcm helicases indicate that they encircle and translocate on one DNA strand while displacing the complemen-

tary strand by exclusion from their central channel (Bochman and Schwacha, 2008), a well-documented mode of helicase action known as the “strand-exclusion” mechanism. Mcm helicases move on single-stranded DNA (ssDNA) in a 3' to 5' direction with the AAA+ ATPase domain proximal to the unwound DNA (McGeoch et al., 2005) (Figure 1A).

Although it is known that pre-RC formation results in the loading of inactive Mcm2–7 helicase onto origin DNA, the nature of this initial association between the helicase and DNA has remained unclear. To address how Mcm2–7 initially associates with DNA upon its loading, Remus et al. reconstitute this helicase loading reaction using purified proteins from the budding yeast *Saccharomyces cerevisiae*. Using electron microscopy to visualize the resulting complexes, the authors make two critical observations. First, although Mcm2–7 in solution is a hexamer prior to loading, the loaded complexes exclusively form double hexamers. Further analysis demonstrates that the double hexamers are oriented head to head with the C-terminal AAA+ domains on the outside and the N-terminal domains at the hexamer-hexamer interface. Second, unlike other hexameric helicases that associate with ssDNA, the authors demonstrate that the loaded Mcm2–7 double hexamers encircle dsDNA. Consistent with this topology, the authors find that the loaded double hexamers slide along dsDNA in the absence of helicase activity. Although viral DNA helicases also form double hexamers (Schuck and Stenlund, 2005), their assembly is associated with DNA melting and distortion, suggesting that

they interact with ssDNA. Such changes in DNA structure are not observed during Mcm2–7 loading. Interestingly, no individual hexamers are observed on the DNA, strongly suggesting that both hexamers are loaded simultaneously.

The mechanism by which the Mcm2–7 complex unwinds DNA in the cell remains unclear. However, the authors' insights into the initial association of the Mcm2–7 helicase with DNA place constraints on how easily different mechanisms could be achieved. If the Mcm2–7 helicase functions by strand exclusion, then substantial remodeling of the loaded Mcm2–7 double hexamer would be required (Figure 1D). This transition would involve melting of the dsDNA, opening of the Mcm2–7 ring, and ejection of the nonengaged DNA strand from each hexamer. Because each Mcm2–7 hexamer is thought to encircle 34 bases of DNA (Remus et al., 2009), an extensive region of melted DNA would be required to create a large enough DNA loop to escape the ring. If the Mcm2–7 central channel opens to allow ssDNA to escape during activation, it must occur without Cdc6. Cdc6 is essential to the requisite Mcm2–7 ring opening during pre-RC formation but it is absent in S phase.

The initial loading of the Mcm2–7 complex onto dsDNA coupled with previous studies demonstrating that the archaeal Mcm helicases can translocate unidirectionally on dsDNA (Shin et al., 2003) support two additional mechanisms of DNA unwinding. Mcm2–7 could unwind DNA using a “ploughshare” mechanism, in which the enzyme forces dsDNA toward a protein “pin” that separates the DNA strands as they exit the hexamer (Taka-

hashi et al., 2005) (Figure 1D). This mechanism would require initial melting of the DNA to allow the binding of a protein pin between the two strands. The pin might be provided by Mcm2–7 itself (after DNA melting and enzyme activation) or by a newly bound protein. Upon entry into S phase, the Mcm2–7 helicase is activated by binding of additional replication factors (Sclafani and Holzen, 2007). Notably, the GINS complex and the Cdc45 protein interact with Mcm2–7 during S phase, and the resulting complex has robust helicase activity (Moyer et al., 2006). Interestingly, the GINS complex preferentially binds ssDNA (Boskovic et al., 2007) and, therefore, represents a candidate to perform the pin function.

If the double hexamer is retained during unwinding, a third mechanism is possible. In this “twin-pump” model, each Mcm2–7 hexamer would promote DNA unwinding by simultaneously pumping dsDNA toward the double hexamer interface, resulting in the extrusion of ssDNA at the interface (Figure 1D). The mechanism of ssDNA translocation by the Mcm2–7 hexamer indicates that ssDNA moves into the central channel at the AAA+ domain side and exits at the N-terminal side (Figure 1A). Assuming that dsDNA enters and exits the Mcm2–7 hexamer in the same way, the orientation of the Mcm2–7 hexamers within the double hexamer would be consistent with a twin-pump model. This model provides the simplest mechanism for Mcm2–7 helicase activation, given that the complex is already associated with the correct template and no prior unwinding of the DNA would be required. Still unclear is how the resulting ssDNA would exit the double hexamer. Even if this mechanism were not used for DNA unwinding during elongation, it could provide an initial means to melt the origin DNA, which would be required to provide ssDNA substrate for the other two unwinding mechanisms. In addition, melting by this mechanism would provide a simple way to unwind the DNA between the two Mcm2–7 active sites, which would not be engaged by either active site of the double hexamer if the two hexamers separated (Figure 1C).

These different models could also influence our view of replication. For example, the twin-pump model would

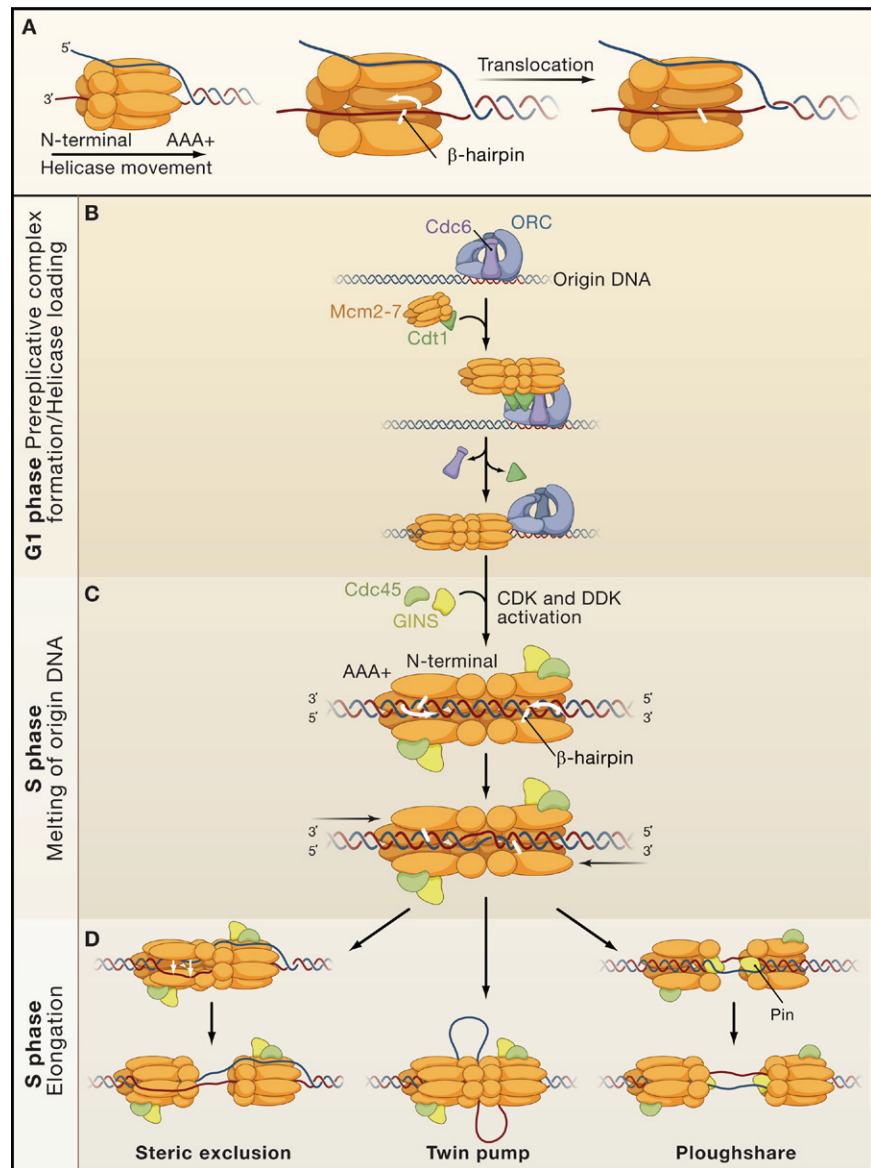


Figure 1. Helicase Loading and Action

(A) Mechanism of Mcm helicase translocation. (Left) The movement of a Mcm2–7 hexamer on single-stranded DNA (ssDNA) displaces the complementary DNA strand. Note that the AAA+ lobe is proximal to the unwound DNA. (Right) The β -hairpin structures within the active sites of each Mcm subunit are thought to change their position and interaction with ssDNA based on the nucleotide bound to the Mcm subunit. For simplicity, only one hairpin is illustrated and two subunits have been removed from the Mcm2–7 hexamer.

(B) Prereplicative complex (pre-RC) formation results in the loading of head-to-head (N-terminal-to-N-terminal) double hexamers of Mcm2–7 that encircle double-stranded DNA (dsDNA). This process requires the origin recognition complex (ORC), Cdc6, and Cdt1 proteins. Both Mcm2–7 hexamers appear to load simultaneously. This suggests that two Mcm2–7/Cdt1 heptamers are recruited to ORC and Cdc6 during assembly.

(C) A model for DNA melting at the origin. In S phase, cyclin-dependent kinase (CDK) and the Dbf4-dependent kinase Cdc7 (DDK) promote the recruitment of numerous additional proteins to the Mcm2–7 complex. Of these, Cdc45 and GINS are thought to activate Mcm2–7 for translocation. If the Mcm2–7 hexamers simultaneously translocate dsDNA, the intervening DNA will be unwound. The arrows on the lower image indicate the direction of dsDNA movement and two subunits are removed to allow visualization of the DNA.

(D) Three models of Mcm2–7 helicase action during replication elongation. In the strand-exclusion model, each Mcm2–7 hexamer encircles and translocates on one DNA strand and excludes the complementary strand. Similar to the DNA melting mechanism described above, the twin-pump model proposes that simultaneous translocation of dsDNA by the Mcm2–7 double hexamer drives DNA unwinding. The ploughshare model proposes that DNA unwinding is driven by individual Mcm2–7 hexamers translocating dsDNA toward a protein pin (illustrated as GINS in this model) that is located between the two strands of the exiting DNA. The intermediate image shows the need for ssDNA for the initial binding of the pin (two subunits are removed to allow visualization of the DNA).

intrinsically lead to “replication factories” with DNA moving toward a dual replisome. On the other hand, the twin pump and ploughshare mechanisms could make repairing intrastrand crosslinks difficult given that the helicase complex would presumably arrest with the crosslink in the Mcm2–7 central channel. Only a better understanding of the replication initiation process will distinguish between these models and reveal the mechanism of Mcm2–7 action.

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Transformation Locked in a Loop

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During neoplastic transformation, cells can promote their own growth by activating proto-oncogenes. Reporting in *Cell*, Iliopoulos et al. (2009) now show that in certain cell types, a transient oncogenic signal is sufficient to induce neoplastic transformation and to maintain it through a positive feedback loop driven by the inflammatory cytokine interleukin-6.

To become fully transformed, primary human cells need to bypass several cellular failsafe mechanisms that normally keep cellular growth in check. In addition, transformed cells can become self-sufficient by producing their own growth signals. Activating mutations in proto-oncogenes are a frequent path to cellular neoplastic transformation, and several reports have described the dependence of cancer cells on the oncogene even after transformation (Weinstein and Joe, 2008). However, in this issue of *Cell*, Struhl and colleagues (Iliopoulos et al., 2009) report that transient induction of the Src oncogene in nontransformed human mammary epithelial cells results in production of the cytokine interleukin-6 (IL-6), which drives—and maintains—cells in a transformed state. This is mediated by a positive feedback loop involving IL-6, NF- κ B, the let-7 microRNA (miRNA), and its regulator, LIN28B, resulting in a “snowball” effect.

NF- κ B is a transcription factor that can induce the expression of IL-6, a cytokine that plays a crucial role in the immune response and inflammation. LIN28B is a stem cell factor and RNA binding protein that inhibits processing of the let-7 miRNA (Viswanathan et al., 2008). Changes in let-7 expression have been associated with tumorigenesis. Although links between inflammation and cancer have been suggested, a link between NF- κ B and let-7 has not been reported so far. However, the new work links all of these four players and demonstrates that they cooperate to maintain cellular transformation in response to transient oncogene activation (Iliopoulos et al., 2009).

How transient is the oncogenic signal and how does it work? Iliopoulos et al. expressed a tamoxifen-inducible estrogen receptor-Src (ER-Src) fusion protein in nontransformed human mammary epithelial cells. They show that a 5 min

treatment with tamoxifen is sufficient to drive the cells into the transformed state. Next, they provide evidence that this transient treatment induces expression of the inflammatory cytokine IL-6 in an NF- κ B-dependent manner. Specifically, they show that Src activates NF- κ B, which directly activates the transcription of LIN28B. Indeed, the expression of the mature let-7 miRNA rapidly decreases in response to Src activation, through induction of LIN28B. As a result, IL-6, a direct target of let-7, is derepressed, causing cellular transformation through STAT3, a transcriptional activator that is phosphorylated and activated in response to IL-6 signaling. A positive feedback loop is generated through IL-6, the expression of which is induced by NF- κ B, but IL-6 can itself activate NF- κ B (Figure 1A). This loop explains why the transformed state is maintained even after Src expression is shut off. Confirming this model, the authors report that