The Hexameric Eukaryotic MCM Helicase: Building Symmetry from Nonidentical Parts*

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The separation of the two strands of the DNA duplex is a prerequisite in the propagation or transfer of genetic information during replication, transcription, and repair. The energy-dependent unwinding of DNA involves a reaction catalyzed by enzymes called helicases. Helicases are protein motors that use the energy of NTP hydrolysis to displace one of the DNA strands and to translocate along the complementary strand (1, 2). Serving both of these functions, helicases move along DNA as an integral part of a macromolecular machine that tracks one of the separated strands for hundreds of kilobases without falling off (3). Processivity of these DNA helicases is therefore crucial to the fidelity of information-transferring mechanisms. A distinct feature of processive helicases is their hexameric ring-shaped structure that enables the enzyme complex to encircle DNA. By encircling DNA, these helicases are topologically linked to DNA, allowing them to travel long distances on DNA without dissociation. To date, over 10 processive helicases from various organisms have been extensively studied (for a comprehensive review, see Patel and Picha (4)). Helicases associated with replication machines include the bacteriophage T7 Gp4 (5, 6), the Escherichia coli DnaB (7), and the SV40 large T-antigen (8, 9). In each of these well studied helicases, the hexameric enzyme is composed of six identical subunits arranged in a ring-shaped structure that has a 6-fold symmetry (10). This 6-fold rotational symmetry coupled to the sequential hydrolysis of NTP by subunits of the hexamer is believed to effect conformational changes that drive the helicase along DNA (11). It is no wonder that enzymologists greeted the latest reports on the eukaryotic MCM helicase with astonishment; recent studies revealed that the helicase is a hexameric enzyme consisting of nonidentical subunits. In this review, we will discuss some of the latest findings related to the eukaryotic MCM helicase, the implied symmetry for this asymmetric complex, and the excitement and new challenges that lie ahead.

Hexameric Replicative Helicases

The initiation of DNA synthesis is a highly orchestrated cellular event. In the model systems (E. coli, bacteriophage T7, and SV40 virus) studied, the assembly of a pre-initiation complex precedes initiation of DNA synthesis at replication origins (12). In all of these examples, a hexameric helicase is a major component of the pre-initiation complex. The first step of the initiation process is the melting of the two DNA strands by the binding of the subunits of the hexameric helicase to origin DNA. The separated strands, stabilized by single-strand DNA-binding proteins, then serve as templates for the synthesis of the first nucleotides of the new DNA chains by primase/polymerase. Elongation of the new DNA chains requires further separation of the DNA templates. In every case, the hexameric helicase responsible for the initial melting of the origin DNA is also the processive helicase that unwinds growing forks. The same principles used for the regulation of replication initiation in viruses and prokaryotes also apply to eukaryotes. However, the complexity of this regulated process increases with the increasing sizes of eukaryote genomes and also the number of replication origins used to replicate these genomes. Depending on the eukaryote, the number of replication origins present per genome ranges from hundreds in Saccharomyces cerevisiae to tens of thousands in mammals. In addition to the large number of proteins that are required both for the recruitment and the activation of this helicase, the eukaryote helicase alone appears to consist of six distinct proteins, known as the MCMs. Based on arguments from genetic (13, 14) and cell-free studies (15–17), the same MCM helicase appears to be responsible for the initiation of all origins in a eukaryote genome.

MCM2-7: A Complex of Six Nonidentical Subunits

The MCMs are proteins required for minichromosome maintenance in yeast (18, 19). Of particular interest is a family of six highly conserved proteins, Mcm2, Mcm3, Mcm4, Mcm5, Mcm6, and Mcm7, that are essential for the initiation of DNA synthesis in all eukaryotes (20, 21). The largest conserved domain of these proteins is a region of about 200 amino acids that encodes a NTP binding motif (Fig. 1A) (20). Despite their structural and functional conservation, each of these MCM proteins is essential for growth and specifically for the initiation and elongation of replication forks (14, 22), suggesting that none of the MCM proteins can substitute for the function of another. Studies from yeast to human indicate that the six MCM proteins interact with each other to form predominantly a hexameric complex containing all six MCM proteins but also other smaller subcomplexes (23–25). Chromatin binding (26, 27) and chromatin cross-linking studies (28) suggest that it is the hexameric complex that is recruited to replication origins before the onset of DNA synthesis during early G1 phase. The exact structure of this chromatin-bound hexameric complex is not known. However, the hexameric complex that copurifies with Mcm2 by immunoaffinity chromatography revealed a globular structure containing all six MCM proteins in equimolar quantities (Fig. 1B) (21, 24). This globular complex is devoid of any detectable enzymatic or nucleotide binding activities (24, 29). Activation of the MCM complex at replication origins requires a series of chemical modifications targeting individual MCM proteins during the G1 to S phase transition (30, 31). It is believed that conformational changes coupled to chemical modifications convert the inactive complex to an active, presumably ring-shaped helicase that encircles DNA. Genetic experiments indicate that phosphorylation of Mcm2 by Cdc7-Dbd4, the G1/S kinase, is a required step in this transition (32, 33). In vitro studies in yeast and in human showed that using the hexameric globular MCM...
complex as substrate, Mcm2 is specifically targeted for phosphorylation by Cdc7-Dbf4 (29, 34). Whether a conformational change in the MCM complex is coupled to this phosphorylation event has not been determined. Genetic evidence suggests that in addition to phosphorylation by Cdc7-Dbf4, modifications of the MCM complex by S phase cyclin-dependent kinases may also be required for the conversion of the inactive MCM complex to the active helicase (Fig. 1B) (30, 31, 35).

The Hexameric MCM Helicase

Evidence for helicase activity associated with the MCM complex was first demonstrated during purification of the hexameric complex from HeLa cells (25). The hexameric complex containing all six MCM proteins is inactive. However, during the course of purification, a trimer of Mcm4, Mcm6, and Mcm7 was generated and a hexamer containing two trimers assembled spontaneously to form a ring-shaped structure (Fig. 1B) (4). This double trimer is associated with ATPase and helicase activity that can unwind about 30 base pairs. Since then, this observation has been confirmed by reconstituting the double trimer from recombinant mouse and Schizosaccharomyces pombe MCM proteins co-expressed in insect cells (36, 37). This double trimer, containing three of the six MCM proteins, shows ATP-dependent ssDNA1 binding, ssDNA-stimulated ATPase activity, and weak 3’→5’ helicase activity. Mutations in the NTP binding motif of Mcm6 and Mcm4 destroyed the helicase and ssDNA binding activity, respectively (36). No other combination of MCM proteins yielded detectable enzymatic activities. In fact, incubation of the double trimer of Mcm4, -6, and -7 with either Mcm2 or the dimer of Mcm3 and Mcm5 resulted in disintegration of the double trimer and inactivation of the helicase (37). Based on these biochemical studies, it was postulated that the active form of the MCM helicase is a hexamer containing only three of the six MCM proteins, which form the catalytic core (4). In this hypothesis, Mcm2, Mcm3, and Mcm5 are regulatory subunits that negatively regulate the activity of the MCM helicase. Activation of the inactive MCM complex to an active helicase requires the removal of Mcm2, Mcm3, and Mcm5 presumably by the phosphorylation of Mcm2 by Cdc7-Dbf4. The nonprocessive helicase activity seen in vitro was attributed to missing acces-
Dinners of Trimers and Hexamers

How do we reconcile the apparent discrepancies between biochemical and genetic studies? One interpretation of these contrasting results is that the in vitro assembled MCM helicase resembles but is not a true representation of the native MCM helicase assembled in vivo. In other words, the double trimer of Mcm4, -6, and -7 is able to assemble into a ring structure in vitro without undergoing the chemical modifications required of the Mcm2–7 hexamer in vivo. This is possible if the in vitro assembled enzyme retains some of the most important features of the native helicase, such as the catalytic core. If so, perhaps there are lessons to be learned from the in vitro assembly of an imperfect but functional enzyme that can be applied to the assembly of the native enzyme. Another interpretation is that the eukaryotic MCM helicase assembled from a double trimer of Mcm4, Mm6, and Mm7 may be involved in other functions such as transcription. A recent report suggests that the MCM proteins are components of the RNA polymerase II holoenzyme (43). Based on in vivo studies (14), the replicative MCM helicase, in contrast, is comprised of all six MCM proteins, and activation of this complex requires multiple steps of modification, culminating in the initiation of DNA synthesis. This precise ritual of events is in keeping with the scheme to ensure that initiation of DNA synthesis occurs no more than once per cell cycle, an elaboration not required for transcription.

A Symmetric Hole from Asymmetric Parts

Some of the more difficult challenges in visualizing the assembly of a hexameric helicase from six nonidentical subunits are, first, how to assemble a symmetric ring structure from six nonidentical parts and, second, how to choreograph such an assembly. That all six MCM proteins are required for the elongation of replication forks in vivo and that there is only one combination of subunits that allows the reconstitution of an active enzyme in vitro suggest that the assembly of the native helicase is ordered and the neighbors are invariant. The conserved central regions of the MCM proteins suggest that it is feasible to build a hexameric ring structure with symmetry in the central channel and at the interface of the subunits from these nonidentical building blocks. As the conserved domains may provide the symmetry for the central channel, the nonconserved N- and C-terminal domains of the individual MCM protein may provide specificity for neighbor interactions (Fig. 1A).

Although all known hexameric helicases form ring-shaped structures with a 6-fold symmetry, kinetic studies of the E. coli Rho and T7 Gp4 helicase showed that not all six subunits are functionally identical (44–46). The subunits exhibit different affinity for NTP binding and different rates for NTP hydrolysis. In both cases, three subunits appear to be catalytic and three noncatalytic. How can a homohexamer contain two types of nucleotide binding sites that display different NTPase properties? The simplest explanation is to propose that subunits of a homohexamer contain alternating catalytic and noncatalytic NTP binding sites such that the enzyme has a 3-fold functional symmetry but a 6-fold structural symmetry. In T7 Gp4, the nucleotide is bound at the subunit interface (6), and thus slight conformational changes at the subunit interface could result in NTP binding sites with different NTPase properties. An analogy was drawn between the homohexameric helicase of Gp4 and the heterohexameric F1-ATPase (45). The F1-ATPase is an $\alpha_3\beta_3$ enzyme that has noncatalytic $\alpha$ subunits (47). The MCM helicase containing six highly conserved but nonidentical subunits provides the prerequisite asymmetry suggested by such a model. Given this scenario, it is not difficult to imagine that Mcm4, Mm6, and Mm7 are the catalytic subunits of a triad that provides the 3-fold functional symmetry in a ring-shaped hexamer (Fig. 1B). In fact, this conceptual model can be used as the basis for the assembly of a hexameric ring that contains only three or all six of the MCM proteins.

Key to Activity: Preservation of the Catalytic Core

A plausible model for the assembly of a ring-shaped hexameric MCM helicase that contains three or six nonidentical subunits through the fusion of two staggered trimers is presented in Fig. 1B. In this model, assembly of the native enzyme involves two nonidentical trimers, one containing the catalytic subunits (Mcm4, Mm6, Mm7) and another containing the regulatory subunits (Mcm2, Mcm3, Mcm5). The fusion of the two staggered trimers gives rise to a hexamer that has the subunits of one trimer interdigitated with those of the other. Conversion of the inactive globular structure to the active ring structure presumably involves multiple posttranslational modifications of the MCM complex including the phosphorylation of Mcm2 by Cdc7-Dbf4 (29, 32, 34). In the in vitro assembly of an active helicase, two staggered trimers of Mcm4, -6, and -7 coalesce to form a ring-shaped hexamer that contains the same triad of subunits in the catalytic core as the native enzyme (Fig. 1B). However, in this case, the regulatory subunits are replaced by the redundant Mcm4, -6, and -7 subunits, which are there to fill a structural rather than a catalytic or a regulatory role in the fortuitous enzyme. The weakened enzyme may have compromised cooperativity as a consequence of the loss of the
regulatory subunits of the native enzyme. This hypothesis predicts that catalytically active hybrid hexamers can be reconstituted by mixing genetically or chemically modified, inactive Mcm4, -6, and -7 trimers with active Mcm4, -6, and -7 trimers.

**Future Directions**

The “merged trimers” model is consistent with all of the in vitro studies on MCM subcomplexes (21). For example, the only stable trimer isolated from cell extracts or during protein purification is Mcm4, -6, and -7 (25, 48), and as a result, only one outcome is observed in the in vitro assembly of an active helicase. The inhibitory effect of Mcm2 on helicase activity (25, 36, 37) can be explained by the stable tetramer formed between Mcm2 and the Mcm4, -6, and -7 trimer (49); and tetramers are nonproductive intermediates in the merged trimers assembly pathway. The model proposed here is speculative but testable.

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