Initiating DNA synthesis: from recruiting to activating the MCM complex

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Summary

The exact duplication of a genome once per cell division is required of every proliferating cell. To achieve this goal, eukaryotes adopt a strategy that limits every replication origin to a single initiation event within a narrow window of the cell cycle by temporally separating the assembly of the pre-replication complex (pre-RC) from the initiation of DNA synthesis. A key component of the pre-RC is the hexameric MCM complex, which is also the presumed helicase of the growing forks. An elaborate mechanism recruits the MCM complex to replication origins, and a regulatory chain reaction converts the poised, but inactive, MCM complex into an enzymatically active helicase. A growing list of proteins, including Mcm10 and Cdt1, are involved in the recruitment process. Two protein kinases, the Cdc7-Dbf4 kinase (DDK) and the cyclin-dependent kinase (CDK), trigger a chain reaction that results in the phosphorylation of the MCM complex and finally in the initiation of DNA synthesis. A composite picture from recent studies suggests that DDK is recruited to the pre-RC during G_1 phase but must wait until S phase to phosphorylate the MCM complex. CDK is required for the recruitment of Cdc45 and other downstream components of the elongation machinery.

Key words: DNA replication, MCM helicase, Mcm10, Cdt1/DUP, CDK, Cdc7-Dbf4

Introduction

The eukaryote genome is organized into multiple chromosomes, whose replication must be coordinated so that one genome equivalent of DNA is precisely duplicated during S phase of every cell division cycle. In metazoans, the rate of DNA replication is regulated at the initiation step by the judicious activation of an appropriate number of initiation events proportional to the rate of cell division at a particular stage of development. In protozoans, the same judicious process for the activation of replication initiation events also takes place, although the mechanism may be less elaborate because protozoans do not have complex developmental programs. The issues that a cell must contend with in preparation for a new round of DNA synthesis are where, when and how initiation events should be targeted such that every chromosome will be replicated exactly once within the allotted time. The concept that chromosomes must be properly groomed during G_1 phase as a preamble to DNA synthesis in S phase was first suggested by mammalian cell fusion studies (Rao and Johnson, 1970). Since then, this concept has been refined in the form of the replication-licensing model (Blow and Laskey, 1988) and more recently articulated in molecular terms as defined steps in the assembly of complexes at replication origins (Diffley et al., 1994). Studies using different model systems suggest that this highly regulated process, which involves at least 20 protein factors, is largely conserved from yeast to humans, and a key player in this regulation is the hexameric MCM complex (Tye, 1999a). MCMs are minichromosome maintenance proteins identified initially for their role in plasmid replication (Maine et al., 1984; Sinha et al., 1986; Tye, 1999a) or cell cycle progression (Kearsey et al., 1995; Moir and Botstein, 1982). Six of these proteins, Mcm2-Mcm7, are highly conserved, interacting with one another to form a complex that plays a direct role in the initiation of DNA synthesis at replication origins (Chong et al., 1995; Kubota et al., 1995; Tanaka et al., 1997; Yan et al., 1993). Recent studies suggest that this MCM complex also serves as a replicative helicase (Labib et al., 2000; Lee and Hurwitz, 2000; You et al., 1999). A simplistic view of the regulation of chromosome replication can be summarized as the regulation of the recruitment and the activation of the MCM complex at replication origins (Tye and Sawyer, 2000). To ensure that initiation occurs only once every cell division, the recruitment of the MCM complex is temporally separated from its activation, when the poised but inactive MCM complex is converted into an enzymatically active helicase. Once activated, the MCM helicase can leave its assigned post, rendering replication origins unable to initiate until the next resetting at G_1 phase. This two-step process is coordinated by an assembly of protein factors that either prepare replication origins for the recruitment of the MCM complex or form part of a chain reaction that culminates in the activation of the MCM complex (Tye, 1999a).

Progress in the past several years has unraveled some of the underlying principles of this temporally regulated process. Briefly, replication origins oscillate between two chromatin states: a pre-replication state (pre-RC), when the MCM complex is localized at replication origins; and a post-replication state (post-RC), when the MCM complex is delocalized from replication origins (Diffley et al., 1994). Serving as scaffold for this oscillating chromatin structure is the origin-recognition complex (ORC) (Bell and Stillman,

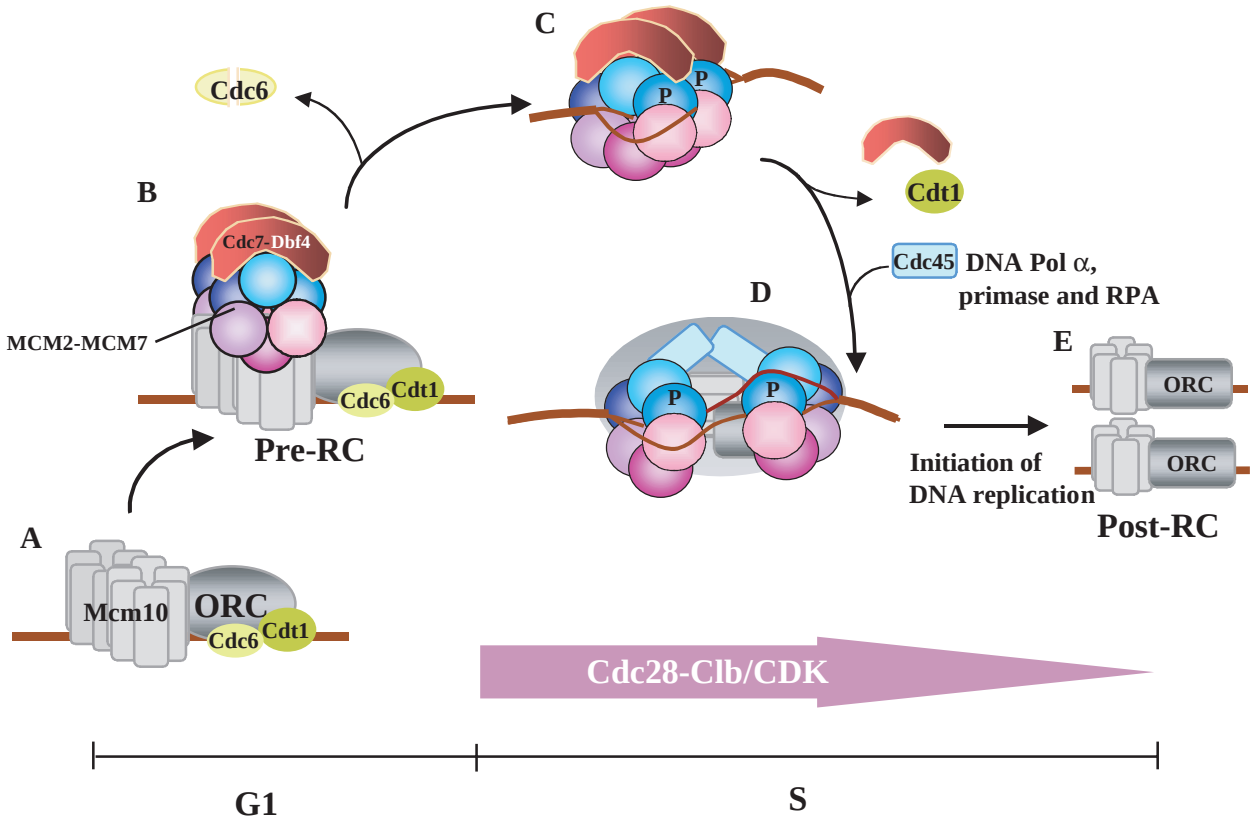

Fig. 1. Initiating DNA synthesis, from recruitment to activation of the MCM complex. (A) Assembly of the pre-RC begins during the G_1 phase, when Cdc6 and Cdt1 are recruited to replication origins to which ORC and Mcm10 bind. (B) Cdc6 and Cdt1 facilitate the loading of the MCM complex. Anchoring of the MCM complex is mediated through interaction between individual subunits of the MCM complex and multimers of the Mcm10 protein. Cdc6 is removed from origins once the MCM complex is recruited. The Cdc7-Dbf4 kinase is also recruited to the origin during G_1 phase. (C) Phosphorylation of the MCM complex by Cdc7-Dbf4 occurs during S phase and is controlled locally at individual origins. Phosphorylation of the MCM complex is coupled to a conformational change in the complex that results in the melting of origin DNA. ORC and Mcm10 are hidden from view. (D) This conformational change is believed to convert the inactive MCM complex into the enzymatically active helicase, whose ring-shaped structure becomes topologically linked to DNA (Tye and Sawyer, 2000). Recruitment of Cdc45 requires both the phosphorylation of the MCM complex and the activity of CDKs. Disassociation of the MCM helicase by Cdc45 from the Mcm10 anchor initiates the melting of DNA and recruits RPA, DNA polymerase α, and primase to the origins for the initiation of DNA synthesis. (E) The melting of the dsDNA induces a conformational change in ORC before replication origins assume a post-replication chromatin state. Like the ORC, Mcm10 is believed to remain bound to origins throughout the cell cycle.

1992). Additional factors, such as the recently identified Mcm10, are also likely to be components of this scaffold (Homesley et al., 2000). Resetting of origins to the pre-RC state takes place during G_1 phase. This window of opportunity is defined by the availability of Cdc6 (Piatti et al., 1996; Piatti et al., 1995) and Cdt1, two chaperone or loading factors for the MCM complex. Cdc6 and Cdt1 facilitate recruitment of the MCM complex indiscriminately to all replication origins such that all potential initiation sites are licensed to replicate (Santocanale and Diffley, 1996; Walter and Newport, 1997). However, not all licensed origins are called into action at the same time. Initiation of DNA synthesis at individual origins appears to be locally controlled, the earliest events taking place at the G_1-to-S phase transition and later events taking place throughout S phase (Bousset and Diffley, 1998; Donaldson et al., 1998). Here, we review recent developments that have contributed to our current understanding of the molecular details of the recruitment and activation of the MCM complex, which is critical for restricting DNA replication to once per cell cycle. We highlight two recently identified MCM-complex-recruiting factors, Mcm10 and Cdt1, and the role of the cyclin-dependent kinases (CDKs) and Dbf4-dependent kinase (DDK), in ordering the sequence of events that precedes the activation of the MCM helicase (Johnston et al., 1999; Nasmyth, 1993; Sclafani, 2000).

Site selection for pre-replication chromatin assembly is determined by ORC binding

Despite divergence of the DNA elements that define replication initiation sites in eukaryotes, the protein factors and the molecular mechanism for the initiation of DNA synthesis appear to be highly conserved in all eukaryotes (Spradling, 1999). Studies of yeast and *Xenopus laevis* model systems have produced a general consensus about the sequence of events that precedes the initiation of DNA synthesis. *Saccharomyces cerevisiae*, which has defined 100-200-bp replication origins provides the simplest model system (Marahrens and Stillman, 1992; Walker et al., 1991). In this unicellular fungus, replication origins are organized in a specialized chromatin

structure around the ORC, which binds to an 11-bp element known as the ARS consensus sequence (ACS) (Bell and Stillman, 1992; Diffley and Cocker, 1992). The binding of ORC and other factors modulates local chromatin structure to facilitate initiation (Lipford and Bell, 2001; Thoma and Simpson, 1985). The ORC binds both double- and single-stranded DNA but exhibits different properties under these different conditions. Binding of ORC to dsDNA is specific to the ACS and inhibits the ORC ATPase activity. In contrast, binding to ssDNA is sequence nonspecific and stimulates ATP hydrolysis. Electron microscopy shows that ORC alternates between two conformations: an extended conformation, when bound to dsDNA; and a bent conformation, when bound to ssDNA (Lee et al., 2000). These observations are consistent with the model that ORC undergoes conformational changes as origin DNA unwinds during the initiation of DNA synthesis, when the pre-replication chromatin becomes post-replication chromatin (Fig. 1B,E; Diffley and Cocker, 1992).

Timing the recruitment of the MCM complex by Cdc6 and Cdt1

Recruitment of the MCM complex (replication licensing) is restricted to a defined period of the cell cycle after exit from mitosis but before the initiation of DNA synthesis. This window of opportunity is afforded by two sets of events: the destruction of mitotic regulators by the anaphase-promoting complex (APC; also known as the cyclosome; Nurse, 1990; Zachariae and Nasmyth, 1999); and the accumulation of G_1-phase-specific initiation factors, including Cdc6 (Cdc18 in *Schizosaccharomyces pombe*) and Cdt1 (DUP in *Drosophila melanogaster*; Blow and Tada, 2000; Maiorano et al., 2000b; Nishitani et al., 2000; Whittaker et al., 2000). Among eukaryotes, there are some minor variations of this theme. In protozoans, destruction of mitotic cyclins by the APC is sufficient to signal the exit from mitosis and the assembly of the pre-RC (Noton and Diffley, 2000). In metazoans, additional negative regulators, including geminin, must be removed by the APC before the assembly of the pre-RC (McGarry and Kirschner, 1998). Geminin negatively regulates Cdt1 function by binding Cdt1 specifically to prevent the recruitment of the MCM complex to replication origins until anaphase (Tada et al., 2001; Wohlschlegel et al., 2001).

Cdc6/Cdc18 and Cdt1/DUP act synergystically to recruit the MCM complex to replication origins. Both Cdc18 and Cdt1 were initially identified as essential factors for DNA replication in *S. pombe* and were noted for their periodic expression under the control of the transcription factor Cdc10 (Hofmann and Beach, 1994; Nasmyth and Nurse, 1981; Zhou and Jong, 1990). Previous studies have shown that constitutive overexpression of Cdc18 leads to the continual synthesis of DNA without mitosis, which causes over-replication of the genome – as expected if resetting of replication origins takes place throughout S phase (Nishitani and Nurse, 1995). The role of Cdc6/Cdc18 in the recruitment of the MCM complex has previously been the subject of intense study; however, the role of Cdt1/DUP in a concurrent step became apparent only recently (Maiorano et al., 2000b; Nishitani et al., 2000). Coexpression of Cdt1 and Cdc18 reduces the threshold required for Cdc18 to induce over-replication (Nishitani et al., 2000). Indeed, studies in *S. pombe* and *X. laevis* showed that Cdc18 and Cdt1 physically interact and both associate with chromatin during G_1 phase in an ORC-dependent manner (Fig. 1A and B). Like ORC, both factors are required for recruitment of the MCM complex but can be removed by salt wash without affecting the retention of the MCM complex in the pre-RC (Hua and Newport, 1998; Rowles et al., 1999; Maiorano et al., 2000a; Nishitani et al., 2000). These observations suggest that Cdc18 and Cdt1 are required for the establishment but not the maintenance of the pre-RC. Like Cdc6/Cdc18, Cdt1 is evolutionarily conserved in eukaryotes (Whittaker et al., 2000), although a Cdt1 homolog in *S. cerevisiae* has yet to be identified.

Anchoring of the MCM complex by Mcm10 in the pre-replication chromatin

The six MCM proteins (Mcm2-Mcm7) interact with one another to form multiple complexes; however, the predominant form purified from *S. pombe* and *Xenopus* egg extracts is a hexamer containing all six MCMs (Adachi et al., 1997; Brown and Kelly, 1998; Kubota et al., 1997). The hexameric MCM complex is relatively stable: all six proteins copurify with Mcm2 in equimolar quantities by immunoaffinity chromatography. Electron microscopy indicates that this complex has a globular structure (Adachi et al., 1997). In vivo studies suggest that all six MCMs are recruited to replication origins during the G_1 phase (Labib et al., 2000; Tanaka et al., 1997). In vitro replication analyses using *Xenopus* egg extracts demonstrate that only the hexamer possesses licensing activity (Maiorano et al., 2000a; Prokhorova and Blow, 2000). However, the hexameric complex lacks enzymatic activity. There is no detectable ATPase, helicase or DNA-binding activities associated with this hexameric complex or with the individually purified recombinant MCM proteins. If neither the hexameric MCM complex nor the individual MCM proteins bind DNA, then what anchors the MCM proteins at replication origins during the assembly of the pre-RC? Even though the recruitment of the MCM complex depends on ORC, Cdc6 and Cdt1, physical contact between these proteins and the MCMs has yet to be demonstrated. Several reports indicate that once the pre-RC is established, the ORC, Cdc6 and Cdt1 can be removed without affecting the retention of the MCM complex or the subsequent initiation activity of the remaining ensemble (Donovan et al., 1997; Hua and Newport, 1998; Maiorano et al., 2000b; Rowles et al., 1999). Therefore, these proteins are unlikely to be responsible for maintaining the association of the MCM complex with replication origins. The manner in which the MCM complex associates with replication origins after the action of Cdc6 and Cdt1 remains a subject of speculation. Because of similarities in sequence between Cdc6 and RF-C, the clamp loader of PCNA (Koonin, 1993), Cdc6 is believed to facilitate the topological linkage of the MCM complex and DNA by enabling MCM to encircle opposite strands of origin DNA as a hexameric ring (Lee and Bell, 2000). However, other studies indicate that there is little change in the footprint of proteins bound to replication origins before or after the loading of the MCM complex by Cdc6 (Perkins and Diffley, 1998). Furthermore, the melting of origin DNA is detected only at a later step when the MCM complex is phosphorylated by Cdc7-Dbf4 at the G_1-to-S phase transition (Geraghty et al., 2000; Lei et al., 1997). These studies suggest

an anchoring mechanism other than a direct contact with DNA for the MCM complex immediately after its recruitment to replication origins.

Two factors, in addition to Cdc7-Dbf4 (Lei et al., 1997), are known to interact with the Mcm2-Mcm7: Mcm10 (Merchant et al., 1997) and Cdc45 (Hopwood and Dalton, 1996). Cdc45 interacts with the Mcm2-Mcm7 in S phase after the assembly of the pre-RC (Zou and Stillman, 1998). Mcm10 was originally identified in a screen similar to that used for the identification of other *mcm* mutants. Homologs of Mcm10 can be found from yeast to humans (Homesley et al., 2000; Izumi et al., 2000). Mcm10 is an abundant chromatin protein that interacts with all six subunits of the Mcm2-Mcm7 complex (Fig. 1B; Homesley et al., 2000; Kawasaki et al., 2000; Merchant et al., 1997). Chromatin immunoprecipitation experiments indicate that it is localized at replication origins (Homesley et al., 2000; W. H. Chai and B. K. Tye, unpublished). These properties of Mcm10 suggest that it is a component of the pre-RC. Indeed, removal of Mcm10 from chromatin after the establishment of the pre-RC during G_1 phase results in the dissociation of the MCM proteins from chromatin without affecting the binding of ORC. Conversely, removal of ORC from the pre-RC does not affect the binding of Mcm10 to chromatin. These and other observations (Coleman et al., 1996) suggest that the recruitment of the MCM complex is dependent on both Mcm10 and ORC, but that the association of the inactive MCM complex with replication origins is mediated by Mcm10 (Homesley et al., 2000). Further studies should assess the role of Mcm10 in the establishment of the pre-RC.

The relationship between Mcm10, Mcm7 and Cdc45

Some of the best insights into the physical interactions between the Mcm2-Mcm7 complex, Mcm10 and Cdc45 came from the unexpected observation that *mcm10-1* is suppressed by mutations in the genes that encode two subunits (Mcm5 and Mcm7) of the Mcm2-Mcm7 complex (Homesley et al., 2000). The suppression of *mcm10-1* by *mcm7-1* or by *mcm5-461* is reciprocal and allele specific. Interestingly, these mutations were initially identified as suppressors of *cdc45-1*, a mutant isolated from an unrelated screen (Moir et al., 1982; Hennessy et al., 1991). Furthermore, characterization of the *mcm10-1 mcm7-1* double mutant showed that all of the defects exhibited by each of the single mutants are corrected in the double mutant, including inviability at 37°C and replication initiation defects. The *mcm10-1* mutant also exhibits a phenotype not observed in other replication initiation mutants: it induces pausing of passing forks at origins that have not initiated, and this fork stalling is alleviated in the double mutant. Most importantly, the double mutant restores the interaction between Mcm10 and Mcm7 that is disrupted in the single mutants. These results suggest that physical contact between Mcm10 and Mcm7 is required for replication initiation as well as smooth passage of elongation forks at unfired origins. Because Mcm10 has been shown also to interact with Mcm2, Mcm3, Mcm4 and Mcm6 (Merchant et al., 1997), the requirement for appropriate contacts between Mcm10 and Mcm7 probably extends to all other subunits of the Mcm2-Mcm7 complex (see Fig. 1).

Mutual suppression that requires conformational changes of two interacting proteins is rare and is observed only under selective pressure. The mutual suppression of *mcm10-1* by *mcm7-1* and *mcm5-461*, two independently isolated suppressors of *cdc45*, suggests that Cdc45 and Mcm10 function in the same pathway. What is the functional relationship between Cdc45 and Mcm10, both partners of the MCM complex? The interaction between Mcm10 and the MCM complex must occur during G_1 phase, when the MCM complex is recruited to the replication origin. The interaction between Cdc45 and the MCM complex occurs sometime at the beginning of S phase just before the migration of the MCM complex away from the replication origin (Aparicio et al., 1999; Zou and Stillman, 1998). An attractive scenario for the three-way interaction is that Cdc45 disengages the MCM complex from its anchor Mcm10 at the critical point when the MCM complex is being converted into an active helicase (Fig. 1D).

Activation of the MCM complex by DDK and CDKs

Activation of the MCM complex is regulated by the S phase CDKs (Nasmyth, 1993) and the Dbf4-dependent kinase (DDK; Jackson et al., 1993; Kitada et al., 1992; Yoon et al., 1993). The catalytic subunits of these kinases are synthesized throughout the cell cycle at relatively low levels. However, their activities are regulated by the periodic synthesis and destruction of their respective regulatory subunits, the S phase cyclins (Dahmann et al., 1995) and Dbf4 (Cheng et al., 1999; Ferreira et al., 2000; Oshiro et al., 1999). Whereas the CDK functions as a global S-phase promoting factor, DDK acts locally to implement initiation at individual replication origins (Pasero et al., 1999). This local regulation, modulated by additional protein kinases such as Rad53, is believed to provide the framework for the temporal regulation of replication initiation at individual origins (Bousset and Diffley, 1998; Donaldson et al., 1998; Kihara et al., 2000; Santocanale and Diffley, 1998; Shirahige et al., 1998; Tanaka and Nasmyth, 1998; Weinreich and Stillman, 1999). Recruitment of the Cdc7-Dbf4 kinase to chromatin is dependent on the MCM complex (Jares and Blow, 2000). However, conflicting results suggesting that Cdc7 and Dbf4 associate with chromatin independently of Cdc6 and of the MCM complex have also been reported (Pasero et al., 1999; Weinreich and Stillman, 1999).

Two important questions concern the activation of the MCM complex by CDK and DDK. What are their target substrates, and do they act sequentially? Although ample evidence supports the involvement of the CDK-cyclin complex Cdc28-Clb in this transitional process (Mimura and Takisawa, 1998; Piatti et al., 1996; Tanaka et al., 1997; Zou and Stillman, 1998), physiological substrates of Cdc28-Clb in the transition of pre-RC to post-RC remain to be identified. Physical contact between Cdc28 and Cdc6 in vitro has been reported (Elsasser et al., 1996), but this interaction may have more to do with the degradation of free Cdc6 than with the origin-bound Cdc6 (Coverley et al., 2000; Jallepalli et al., 1997). Similarly, phosphorylation of MCMs by CDKs has been reported but only in the context of the negative regulation of re-initiation (Hendrickson et al., 1996; Ishimi et al., 2000; Labib et al., 1999).

The target of the Cdc7-Dbf4 kinase is better defined. The first evidence indicating that the MCM proteins may be targets

of regulation by Cdc7-Dbf4 came from genetic studies in *S. cerevisiae*. A mutation that bypasses the requirement for Cdc7-Dbf4 was identified as *mcm5-bob1*, a mutation in *MCM5* (Hardy et al., 1997). Furthermore, *dbf4-6* was identified as a suppressor of the lethal phenotype of *mcm2-1*, and this suppression is reciprocal (Lei et al., 1997). Although the Cdc7-Dbf4 kinase phosphorylates several MCM proteins individually in vitro (Lei et al., 1997; Oshiro et al., 1999; Weinreich and Stillman, 1999), the preferred target of phosphorylation of the MCM hexamer is the Mcm2 subunit (Brown and Kelly, 1998; Jiang et al., 1999; Lei et al., 1997). These observations together argue that phosphorylation of Mcm2 is the only essential function of Cdc7-Dbf4 in the initiation of DNA replication. Interestingly, although a mutation in *mcm5* is able to bypass Cdc7-Dbf4 function, Mcm5 is not one of the substrates phosphorylated by Cdc7-Dbf4 in vitro or in vivo (Lei et al., 1997; Oshiro et al., 1999; Weinreich and Stillman, 1999). A plausible explanation for these observations is that phosphorylation of Mcm2 by Cdc7-Dbf4 induces a conformational change in the MCM complex that is equivalent to that induced by the *mcm5-bob1* mutant protein in the MCM complex. If producing this conformational change were the only essential function of Cdc7-Dbf4, then the *mcm5-bob1* mutation would bypass the requirement for Cdc7-Dbf4 (Lei et al., 1997). This hypothesis is supported by the recent observation that origin DNA melts in wild-type cells after the Cdc7-Dbf4 has acted at the beginning of S phase but melts in the *mcm5-bob1* mutant during G_1 phase (Geraghty et al., 2000). Challenges in the future will be to correlate the phosphorylation of the MCM complex by Cdc7-Dbf4 with physical changes in the hexameric MCM complex and to determine whether the local melting of origin DNA is the result of the conversion of the globular complex to the ring-shaped helicase (Fig. 1B,C; Sato et al., 2000; Tye and Sawyer, 2000).

The Cdc7-Dbf4 function is executed in two steps

The sequence of events regulated by Cdc7-Dbf4 and CDKs that results in the initiation of DNA synthesis has been actively investigated in *S. cerevisiae* (Nougarede et al., 2000; Owens et al., 1997; Pasero et al., 1999; Weinreich and Stillman, 1999; Zou and Stillman, 2000) and in *Xenopus* eggs (Jares and Blow, 2000; Walter, 2000). Some studies support the model that CDKs act before Cdc7-Dbf4 (Nougarede et al., 2000); others indicate that Cdc7-Dbf4 acts before CDKs (Walter, 2000). The inherent differences between yeast and *Xenopus* biology might explain these conflicting results (Takisawa et al., 2000; Walter, 2000). However, experimental differences may account for some of the discrepancies. Some differences, for example, can be attributed to the presence of only DDK during G_1 phase in yeast cells but the presence of both CDKs and DDK in *Xenopus* egg extracts.

All of these studies can be brought together into one coherent picture, however, given the following considerations. Cdc7-Dbf4 might execute its function in two, temporally distinct steps: the recruitment of Cdc7-Dbf4 to the pre-RC in G_1 phase; and the phosphorylation of Mcm2 by Cdc7-Dbf4 in S phase. Furthermore, recruitment of Cdc45 is dependent on both the phosphorylation of Mcm2 by DDK and CDK activity (Fig. 2). All of these considerations are supported by experimental observations. Evidence suggests that Dbf4 and Cdc7 are recruited either together or individually to replication origins during G_1 phase (Dowell et al., 1994; Pasero et al., 1999). Cdc7-Dbf4 function at replication origins is restricted to S phase (Bousset and Diffley, 1998; Donaldson et al., 1998; Hereford and Hartwell, 1974; Pasero et al., 1999). Moreover, the interaction between Cdc45 and Mcm2 occurs during S phase, after the Cdc7-Dbf4 kinase has acted, in a CDK-dependent manner (Aparicio et al., 1999; Zou and Stillman, 2000).

If Cdc7-Dbf4 acts at two temporally distinct steps, then reciprocal shift experiments in *S. cerevisiae* should produce the following results independently of CDK activity. Inactivation of the Cdc7-Dbf4 kinase activity during G_1 phase under nonpermissive conditions might not affect the recruitment of Cdc7-Dbf4, and therefore Mcm2 should still be phosphorylated on return to the permissive condition during S phase. However, expression of Cdc7-Dbf4 during G_1 phase, followed by inactivation of the kinase during S phase, even in the presence of CDK, should prevent the phosphorylation of Mcm2. The apparent result is that Cdc7-Dbf4 cannot execute its function without prior CDK action (Nougarede et al., 2000). In the *Xenopus* egg cell-free system, both CDK and DDK are active in interphase extracts. Incubation of DNA template in CDK-depleted extracts should allow recruitment of Cdc7-Dbf4 to the pre-RC and phosphorylation of Mcm2 (Jares and Blow,

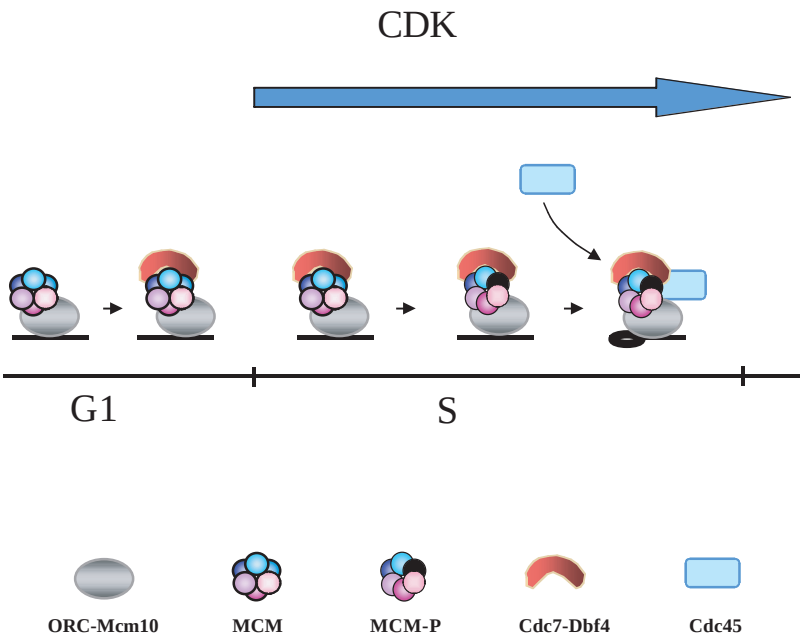

Fig. 2. The Cdc7-Dbf4 kinase executes its essential function in the initiation of DNA synthesis in two temporally separable steps. Recruitment of Cdc7-Dbf4 to replication origins occurs during G_1 phase. However, Cdc7-Dbf4 function must act sometime during S phase, and this decision is controlled locally at individual origins. Phosphorylation of Mcm2 by Cdc7-Dbf4 results in a conformational change in the MCM complex. Recruitment of Cdc45 is dependent on both the phosphorylation of the MCM complex and the activity of the CDK.

2000). When the preassembled template is transferred into extracts containing CDK, Cdc45 is recruited to the phosphorylated Mcm2, allowing initiation to occur. However, when template assembled in Cdc7-depleted extracts containing CDK is transferred into extracts containing DDK, but lacking CDK, Cdc45 cannot be recruited to the pre-RC, regardless of whether Mcm2 is phosphorylated. The apparent result is that DDK must act before CDK in order for Mcm2 to be phosphorylated (Jares and Blow, 2000; Walter, 2000). Thus, reinterpretation of both the yeast and the *Xenopus* egg cell-free studies in this new light will minimize the discrepancies reported by the different groups.

We favor the notion that Cdc7-Dbf4 performs its function at two temporally distinct steps both in yeast and in *Xenopus* eggs, because it underscores the evolutionary conservation of the order of events that activate the MCM complex. This model, however, creates a new quandary. If Cdc7-Dbf4 is recruited to the MCM complex during G_1 phase but can only phosphorylate Mcm2 during S phase at individual origins, then what prevents the phosphorylation of Mcm2 by Cdc7-Dbf4 during G_1 phase? Further studies are needed to differentiate whether experimental design or inherent biology is the culprit for these contradictory observations.

Perspectives

Our knowledge of the regulatory mechanism that ensures the fidelity of genome duplication in eukaryotes has increased by leaps and bounds in the past decade. More than 20 proteins have been identified as essential components that must be pre-assembled at replication origins for initiation to occur. The characterization of Cdt1 and Mcm10 in the past year has provided new insights into the assembly process. A recurring theme in the ordered assembly of complexes that have multiple components is that each of the components must be in place before collective action can begin. This common theme is translated into the temporal separation of complex assembly and the initiation of action, a scheme used to ensure that DNA replication occurs once and only once every cell division cycle. The recruitment of the Cdc7-Dbf4 kinase during G_1 phase and its phosphorylation of Mcm2 during S phase is one example (Fig. 2). The recruitment of the MCM complex during G_1 phase and its subsequent conversion into an enzymatically active helicase during S phase is another example (Fig. 2). Each of these activities is part of a chain reaction that culminates in the initiation of DNA synthesis (Fig. 1). This conceptually simple strategy requires careful coordination on the part of the eukaryote cell. ORC and Mcm10 are delegated to serve as the landing pad for this operation. Cdc6 and Cdt1 prepare the site for the arrival of the MCM complex. Cdc7-Dbf4 is the last to arrive during G_1 phase, after the recruitment of the MCM complex (Jares and Blow, 2000), but has the final control in determining when Mcm2 can be phosphorylated during S phase. We believe that this phosphorylation sets off a chain reaction that results in a conformational change in the MCM complex allowing the recruitment of Cdc45, which both disengages the MCM helicase from its anchor and recruits the elongation machinery. However, the recruitment of Cdc45 and the downstream components cannot take place without the blessing of the CDKs (Mimura and Takisawa, 1998; Walter and Newport, 2000). This picture may seem pleasingly complete on the basis of what we know today but will no doubt be subject to revision when additional players are identified.

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