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Regulation of Minichromosome Maintenance (MCM) Helicase in Response to Replication Stress

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1. Introduction

Progression of DNA replication forks is hindered by various reasons such as DNA-binding proteins, DNA damage, and dNTP depletion. The minichromosome maintenance (MCM) complex, which comprises of Mcm2–7 subunits, is the DNA helicase that plays the central role in the progression of replication forks. MCMs are loaded onto specific sites of chromosomes called origins to create pre–replication complexes (pre–RCs). At the onset of the S–phase, MCM forms a complex with GINS and Cdc45, and starts unwinding the double-stranded DNA providing template strands to polymerases. Even after the fork encounters replication stress, the DNA unwinding continues to some extent and causes an extended length of single-stranded DNA to be exposed, which in turn induces the activation of checkpoint pathways. However, unregulated translocation of MCM may cause re-annealing of two complementary single-strands behind the fork and destabilize the stalled replication machinery. Thus, DNA helicase activity needs to be tightly regulated under the stress conditions in order to preserve the replication machinery. It appears that the proteins associated with the replication forks including Mrc1/Claspin, Tof1/Swi1/Tim1, Csm3/Swi3/Tipin, Ctf4/Mcl1/AND-1 mediate the interaction between the MCM helicase and DNA polymerases, and are required for coupling of DNA unwinding with DNA synthesis. The evolutionally conserved C-terminal domain (CTD) of Mcm4 is also involved in the regulation of the MCM helicase activity. The checkpoint kinase, cyclin-dependent (CDK), Dbf4-dependent kinase (DDK) and Polo-like kinase (PLK) kinase phosphorylate and regulate the function of the MCM helicase. Here we review the recent findings in regard to the MCM regulation in response to replication stress, and discuss how DNA synthesis and unwinding are coordinated to maintain the genome integrity.

2. Formation of the replication machineries on eukaryotic chromosomes

2.1 Assembly of pre-Replication Complex (pre-RC)

A single round of replication of chromosomal DNA in every cell cycle is important for faithful transmission of the genetic information to daughter cells. G1-phase of the cell cycle is marked as the growth phase, which prepares the cells for the S-phase (replication) (Pardee, 1989). The initiation sites of DNA replication on chromosomes are called replication

origins. A series of proteins that are specifically required for chromosome replication, shown in Table 1, are assembled on each origin for the initiation. Essentially, all of the replication proteins are conserved from yeasts to humans, making yeast an excellent model system to understand the molecular mechanisms of DNA replication. The origin recognition complex (ORC), which consists of Orc1-6 subunits, has been identified as the protein complex that binds to the origin DNA in an ATP-dependent manner (Bell & Stillman, 1992). The minichromosome maintenance (MCM) complex, that forms a ring-like structure consisting of Mcm2-7 subunits each of which contains the AAA+ motif (Neuwald et al, 1999), has been originally identified by a genetic screening for the budding yeast mutants that affect autonomous replication sequence (ARS) activity on episomal plasmids (Maine et al, 1984). MCM proteins were independently identified from Xenopus egg extracts as the factor that binds to chromosomes, licensing the initiation of DNA replication (Blow & Laskey, 1986; Kubota et al, 1995). In 1997, DNA helicase activity associated with MCM was first reported by using Mcm4-6-7 sub-complex in an *in vitro* assay (Ishimi, 1997). Using conditional degron system, it has been shown that MCM is essential for the initiation as well as the elongation phases of DNA replication (Labib et al, 2000). From late M to G1 phase, MCMs are loaded onto the origins depending on Cdt1 and Cdc6, resulting in the formation of pre–replicative complexes (pre–RCs) (Diffley et al, 1994; Donovan et al, 1997; Liang et al, 1995; Nishitani et

Budding yeast	Fission yeat	Frog	Mammal
pre-RC components			
Orc1-6 (ORC)	Orc1-6 (ORC)	Orc1-6 (ORC)	Orc1-6 (ORC)
Mcm2-7 (MCM)	Mcm2-7 (MCM)	Mcm2-7 (MCM)	Mcm2-7 (MCM)
Cdc6	Cdc18	Cdc6	Cdc6
Cdt1	Cdt1	Cdt1	Cdt1
Initiation factors			
Sld2	Drc1	RTS/RecQ4	RTS/RecQL4
Sld3	Sld3	Treslin/Ticrr	Treslin
Dpb11	Cut5/Rad4	TopBP1/Cut5/Mus101	TopBP1
Initiation and elongation factors			
Sld5–Psf1-Psf2-Psf3 (GINS)	Sld5-Psf1-Psf2-Psf3 (GINS)	Sld5-Psf1-Psf2-Psf3 (GINS)	Sld5-Psf1-Psf2-Psf3 (GINS)
Cdc45/Sld4	Cdc45/Sna41	Cdc45	Cdc45
Mcm10/Dna43	Mcm10/Cdc23	Mcm10	Mcm10
Luxury components in replication fork			
Tof1	Swi1	TIM1	Tim1
Csm3	Swi3	TIPIN	Tipin
Mrc1	Mrc1	CLASPIN	Claspin/CLSPN
Ctf4	Mcl1	AND-1	AND-1

Table 1. DNA replication proteins are conserved from yeasts to humans.

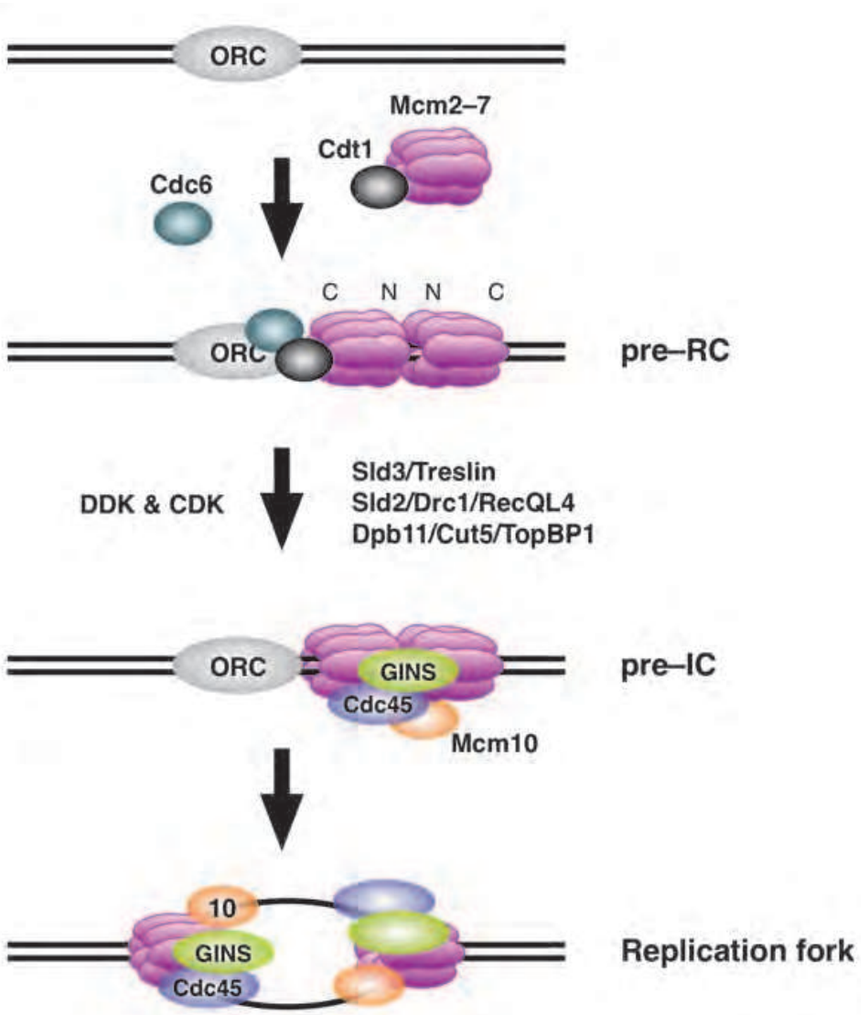

Fig. 1. Stepwise formation of DNA replication forks in eukaryotes.

al, 2000; Remus et al, 2009). The pre–RC is an important landing pad for the replication initiation, because it is prerequisite for the assembly of other factors that are essential for replication. Being connected via their N-terminal domains, head-to-head double hexamers of Mcm2-7 are stably and exclusively formed on the origin DNA (Evrin et al, 2009; Gambus et al, 2011; Remus et al, 2009). Each of the double hexamers may be involved in bi-directional replication from a single initiation site. Although DNA replication initiates from the single replication origin *oriC* in *E. coli* (Kaguni et al, 1982), there are large number of origins where pre-RCs are assembled onto each chromosome of eukaryotes. In both budding and fission yeasts, there are hundreds of origins per genome of about 14-Mb (Hayashi et al, 2007; Wyrick et al, 2001). Under the unperturbed condition, replication starts from only a subset of these origins. The dormant origins serve as the replication initiation sites under the stressed conditions (see below). In multicellular organisms, the origins that fire in early S phase differ during development or between different cell types (Goldman et al, 1984). It is also important for normal development that many pre-RCs are produced in the genome,

because mutations in ORC, Cdc6, or Cdt1 have been found in the patients of Meier-Gorlin syndrome (Bicknell et al, 2011a; Bicknell et al, 2011b; Guernsey et al, 2011), which is a rare autosomal recessive genetic condition whose primary clinical hallmarks include small stature, small external ears and small or absent patellae.

2.2 Assembly of pre-Initiation Complex (pre-IC)

The choice of the origins to be used is determined by the loading of additional replication proteins onto pre-RCs to form pre-initiation complexes (pre-ICs). Among the components of pre-IC, it seems that Sld2/Drc1/RecQ4/RecQL4 (Kamimura et al, 1998; Sangrithi et al, 2005; Wang & Elledge, 1999), Sld3/Treslin (Kamimura et al, 2001; Kumagai et al, 2010; Nakajima & Masukata, 2002; Sansam et al, 2010), and Dpb11/Cut5/Rad4/TopBP1 (Araki et al, 1995; Saka & Yanagida, 1993; Yamane et al, 1997) are essential for the initiation but not for the elongation phase of replication. Two classes of protein kinase that are active in the S phase: Dbf4-dependent kinase (DDK) and cyclin-dependent kinase (CDK) play important roles in the assembly of pre-IC and ensure that only a single round of DNA replication takes place in each cell cycle. Mcm2, 3, 4, and 6 subunits can be phosphorylated by DDK *in vitro* (Lei et al, 1997), and the phosphorylation of Mcm4 appears to be critical for loading of Cdc45 onto pre-RCs (Masai et al, 2006; Sheu & Stillman, 2006; Sheu & Stillman, 2010). In fission yeast, loading of Sld3 onto origins occurs in a DDK-dependent but CDK-independent manner (Yabuuchi et al, 2006). And, Sld3 and DDK are required for the loading of Cdc45 as well as GINS complex (Yabuuchi et al, 2006; Yamada et al, 2004). Thus, it is tempting to argue that the loading of Sld3 is mediated by the phosphorylation of MCM proteins by DDK. It has been established that Sld2 and Sld3 are the two major substrates of CDK-dependent phosphorylation that are essential for replication in budding yeast (Masumoto et al, 2002; Tanaka et al, 2007; Zegerman & Diffley, 2007). The phosphorylation of Sld2 and Sld3 enhances their interaction with Dpb11 that contains multiple copies of the BRCT motif, which is the phosphopeptide-binding module found in many other proteins including BRCA2 (Glover et al, 2004). As both Sld2 and Sld3 are essential for stable association of Dpb11 to replication origins, the phosphorylation-mediated interaction between them is required for the Dpb11 loading onto pre-RCs. The CDK-mediated regulation for the assembly of Sld2 and Sld3 with the BRCT-motif-containing protein, Dpb11/Cut5/TopBP1 appears to be an evolutionally conserved mechanism, as the fission yeast homolog of Sld2, Drc1 is phosphorylated by Cdc2 kinase, and the Drc1 phosphorylation appears to be important for interaction with the homolog of Dpb11, Cut5/Rad4 in a CDK-dependent manner (Fukuura et al, 2011; Noguchi et al, 2002). Furthermore, it has been shown that the mammalian homolog of Sld3, Treslin associates with TopBP1 in a Cdk2-dependent manner and is essential for the initiation of DNA replication (Kumagai et al, 2010). Dpb11 forms a complex with DNA polymerase ε (Masumoto et al, 2000), and is required for the loading of the Sld5-Psf1-Psf2-Psf3 (GINS) complex (Kanemaki et al, 2003; Kubota et al, 2003; Takayama et al, 2003) and Cdc45 (Muramatsu et al, 2010) onto pre-RCs. Mcm10 protein also binds to subunits of Mcm2-7 complex, and is essential for the initiation and elongation of replication, although its mechanism of action remains elusive (Aves et al, 1998; Heller et al, 2011; Izumi et al, 2000; Solomon et al, 1992). In the S phase, MCM proteins and Cdc45 dissociate from origin and travel along DNA, concerted with DNA polymerase ε (Aparicio et al, 1997). The traveling along DNA has also been observed for GINS (Kanemaki et al, 2003; Takayama et al, 2003). Purification of proteins from yeast cells in the S phase identified the complexes

containing Cdc45, GINS and Mcm2-7 (Gambus et al, 2006). *Xenopus* Cdc45, GINS and Mcm2-7 were also identified at the replication fork (Pacek et al, 2006). In vitro experiments showed that the Cdc45/Mcm2–7/GINS (CMG) complexes purified from *Drosophila* cells exhibit robust DNA unwinding activity, and that the CMG complex translocates on DNA in a 3′ to 5′ direction (Moyer et al, 2006). Association with Cdc45 and GINS enhances ATP hydrolysis, DNA binding and the helicase activity of Mcm2–7 (Ilves et al, 2010). These studies show that the CMG complex functions as the DNA unwinding complex in the replication machinery. Replication may begin such that two forks originate from a single origin moving in opposite directions (bi-directionally) behind the CMG complex, which unwinds DNA to provide single-stranded DNA (ssDNA) templates to the polymerases for duplication. To sum up, CMG complex is the motor of the replication fork that unwinds double-stranded DNA (dsDNA), providing template ssDNA for polymerases that synthesize new strands. Therefore, it is important to understand the formation and the regulation of the CMG complex to elucidate the molecular mechanism of DNA replication.

3. Blockage to the progression of DNA replication forks

DNA replication forks may encounter certain impediments such as DNA damage, dNTP depletion, the proteins that tightly bind to DNA, or epigenetic status of nucleosomes. In order to replicate the entire genome within a limited period of time, such aforementioned blocks are necessary to be removed or tolerated. Failures to respond to these replication fork blockages lead to genome instability such as gross chromosomal rearrangements (GCRs), consequently leading to cell death or genetic diseases including cancer in multicellular organisms.

3.1 Inhibitors to the replication fork progression

Replication is an eventful process liable to encounter odds during its procession. Various exogenous substances have been recognized to obstruct this process in their own specific mode of action. Hydroxyurea (HU) is a specific inhibitor of the enzyme ribonucleotide reductase (RNR) that is essential for production of deoxyribonucleotides (dNTPs) (Young et al, 1967). Thus, treatment of cells with HU inhibits DNA synthesis by depleting dNTPs. Aphidicolin is a tetracyclic diterpene tetraol, obtained from *C. aphidicola* and certain other fungi (Bucknall et al, 1973). It is a specific and direct inhibitor of DNA polymerase α and also δ, two of the three DNA polymerases (i.e. DNA Pol α, δ, and ε) that are essential for chromosomal DNA replication (Goscin & Byrnes, 1982; Ikegami et al, 1978). Thus, treatment of cells with either HU or aphidicolin inhibits the progression of replication forks. Chemical adducts on DNA have the potential to impede the fork progression. Methyl methanesulfonate (MMS) is one such well-characterized DNA alkylating agent. The predominant adduct in dsDNA resulting from MMS exposure is 7-methylguanine (*N*7-meG) followed by 3-methyladenine (*N*3-meA) (Lawley & Brookes, 1963). The methylation of DNA physically impedes the progression of replication forks, leading to the formation of DNA double-strand breaks (DSBs) during S phase (Groth et al, 2010). Camptothecin (CPT) is a specific inhibitor of DNA topoisomerase I (Top1) capable of removing DNA supercoils during replication as well as transcription. CPT blocks both the DNA cleavage and re-ligation reactions of Top1 (Kjeldsen et al, 1992). Inhibition of the initial cleavage step leads to the accumulation of supercoils ahead of the replication fork that induces potentially lethal

DNA lesions (Koster et al, 2007). Blocking the rejoining step accumulates the reaction intermediates in which Top1 is covalently attached to the end of DNA (Hsiang et al, 1985). When replication forks reach these DNA nicks, they will be converted to one-ended DSBs. CPT analogues have significant activity against solid tumours, and have gained US Food and Drug Administration approval for the treatment of ovarian and lung cancer (Stewart, 2004). DNA interstrand cross-links (ICLs) also block the fork progression, by preventing the two DNA strands from separating. Antitumor drugs such as cisplatin, psoralen, nitrogen mustard or mitomycin C as well as endogenous agents formed by lipid peroxidation induce ICLs (Niedernhofer et al, 2003). There are different types of chemicals that block the progression of replication forks, and they do so by distinct mechanisms.

3.2 Replication fork pausing sites on chromosomes

Progression of replication forks is affected either by protein-DNA complexes or the torsional stress around the fork. It has been estimated that there are >1,000 discrete sites in the budding yeast genome that impede normal fork progression, including tRNA and rDNA genes, dormant origins, silent mating-type loci, centromeres and telomeres (Ivessa et al, 2003). Non-nucleosomal and nucleosomal protein-DNA complexes impede the fork progression (Deshpande & Newlon, 1996; Ivessa et al, 2003). Replication fork barrier (RFB) present in the rDNA gene is one of the well-characterized pausing sites in the genome. The RFBs block fork progression in an orientation dependent manner. The 3' end of the 35S rRNA transcription unit in rDNA acts as a barrier to replication forks moving in the direction opposite to RNA polymerase I (Brewer & Fangman, 1988). The arrest of replication forks at the RFB site occurs independently of transcription but is mediated by the Fob1 protein that binds to the specific sequence of ~100 bp in the RFB site (Brewer et al, 1992; Kobayashi et al, 1992; Kobayashi & Horiuchi, 1996). However, the Fob1 binding to the RFB site is not sufficient for the transcription-independent fork arrest (Calzada et al, 2005). Tof1, Csm3, and Mrc1 are the luxury members of the replication machinery that are associated with the CMG complex (Katou et al, 2003). Among them Tof1 and Csm3 are required for the pausing at the RFB in rDNA of budding yeast (Calzada et al, 2005). The fission yeast homologs, Swi1 and Swi3, are also required for the fork arrest at the RFB site in rDNA and at another orientation-dependent fork barrier site present in the mating-type switch locus, RTS1 (Dalgaard & Klar, 2000). Thus, it is possible that the proteins that bind to the barrier sites are not simple obstacles to the fork movement rather they negatively regulate the CMG activity via a specific interaction with the fork components.

In the absence of RFB in the rDNA locus, DNA and RNA syntheses simultaneously advance on the same template DNA, the 35S rRNA gene, making a collision between oppositely traveling replication and transcription machineries. When transcription occurs frequently, this collision slows down the progression of replication forks (Takeuchi et al, 2003). The fork pausing by the collision between transcription and replication machineries is also observed in other regions of chromosomes. Some of the highly transcribed tRNA genes and RNA polymerase II genes also impede the progression of replication forks. Genome-wide analyses of DNA Pol2–binding sites, which are indicative of the position of replication forks, showed that the fork arrest takes place regardless of whether replication and transcription move in the same or opposite directions (Azvolinsky et al, 2009). Direction-independent collision was confirmed by similar experiments that mapped the position of the Psf2 subunit of the GINS complex (Sekedat et al, 2010), suggesting that replication forks arrest at highly transcribed genes because the transcription and replication machineries are not allowed to occupy the same DNA at the same time.

DNA supercoils produced ahead of advancing replication forks are resolved by a coordinated action of Top1 and Top2. In the absence of functional topoisomerases, the replication fork-related topological constrains are accumulated, leading to fork collapse and DNA damage checkpoint activation (Bermejo et al, 2007; Brill et al, 1987; Kim & Wang, 1989). Behind the replication fork, intertwining of sister chromatid DNA takes place. Top2 activity is essential for the separation of intertwined chromosomal DNA molecules before the onset of anaphase (Holm et al, 1985). Expression of catalytically inactive Top2 prevents completion of DNA replication and induces DNA damage checkpoint response (Baxter & Diffley, 2008), suggesting that the processing of catenation of sister chromatids behind the fork may also affect the fork progression.

The replication pausing sites are chromosome fragile sites. γ-H2A or γ-H2AX is one of the well-characterized histone modifications that occur around DNA damage sites in the checkpoint kinase dependent manner (Downs et al, 2000). By using DNA microarray, recent studies mapped the localization of γ-H2A on budding or fission yeast genomes in unperturbed S phase (Rozenzhak et al, 2010; Szilard et al, 2010). In budding yeast, they found the accumulation of γ-H2A to occur in repressed genes and that is dependent on the activity of a histone deacetylase (HDAC). In fission yeast, γ-H2A and the Brc1 protein that recognizes γ-H2A through a pair of BRCT domains were localized at heterochromatin regions of chromosomes such as silent-mating type loci, centromeres and telomeres. They also showed that the S-phase specific localization of γ-H2A and Brc1 is dependent on Clr4, which is responsible for the methylation of histone H3 9th Lys. Thus, HDAC- and/or H3K9^{me}-mediated heterochromatin seems to impair the stability of replication forks when they pass through, although how the chromatin status affects the fork progression remains to be elucidated.

4. Checkpoint response to replication problems

Replication problems such as replication fork stalling or collapse are detected by the surveillance system called checkpoint. The replication checkpoint pathway is activated in response to replication stalling, while the DNA damage checkpoint will be activated when DSBs are formed. These checkpoint pathways temporarily halt the cell cycle progression, giving time for cells to solve the replication problems before entering into mitosis.

4.1 Checkpoint activation in response to replication problems

Extended lengths of ssDNA formed at stalled forks is the key DNA structure that induces the replication checkpoint response. The ssDNA never exists naked in vivo and is immediately coated by ssDNA-binding proteins: replication protein A (RPA). The ssDNA–RPA complex recruits the most upstream checkpoint kinase the ATR-ATRIP complex in mammals (Choi et al, 2010; Zou & Elledge, 2003). Primer-template junctions present right next to the ssDNA–RPA complex in the context of stalled forks are also important for the ATR activation (MacDougall et al, 2007). The proliferating cell nuclear antigen (PCNA)–related checkpoint clamp, the Rad9–Rad1–Hus1 (9–1–1) complex is recruited to stalled forks with the aid of the checkpoint-specific clamp loader (Bermudez et al, 2003; Ellison & Stillman, 2003; Zou et al, 2002). Intriguingly, the essential replication initiation protein Dpb11/Cut5/TopBP1 is again recruited to the stalled fork depending on the protein phosphorylation induced by the ATR kinase, and is required for the activation of replication checkpoint (Delacroix et al, 2007; Furuya et al, 2004; Kumagai et al, 2006; Lee et al, 2007),

suggesting a mechanistic similarity between the replication initiation and the checkpoint activation. Tof1/Swi1, Csm3/Swi3, and Mrc1 form the protein complex associated with the CMG complex (Bando et al, 2009; Katou et al, 2003; Noguchi et al, 2004; Shimmoto et al, 2009). Tof1/Swi1 and Csm3/Swi3 form a relatively stable complex and are required for the association of Mrc1 with the replication fork. Although this fork protection complex is dispensable for DNA replication, it is required for the activation of the replication checkpoint effector kinase such as fission yeast Cds1 (Alcasabas et al, 2001; Murakami & Okayama, 1995; Noguchi et al, 2003; Tanaka & Russell, 2001; Unsal-Kacmaz et al, 2007), indicating that the substrate specificity of the most upstream checkpoint kinase is modulated by the proteins associated with the CMG complex.

As mentioned above, the extension of single-stranded region at times of replication stress is crucial to induce a cascade of checkpoint responses. Even after DNA polymerases come to a halt, the CMG complex continues to execute its job as a helicase to a considerable length to produce the single-strand region sufficient to activate the checkpoint. Inhibition of Cdc45 or Mcm7 after the replication initiation blocks accumulation of RPA on chromatin and the checkpoint activation (Byun et al, 2005). Thus, DNA unwinding activity of the CMG complex is required not only for DNA synthesis under unperturbed condition, but also for the activation of the replication checkpoint at times of replication stress. The interactions between the CMG complex and the checkpoint proteins may also contribute to the activation of replication checkpoint (Tsao et al, 2004). There is an intimate link between the CMG complex and the replication checkpoint. However, when stalled forks are collapsed to create DSBs, the damage checkpoint will be activated. The Mre11-Rad50-Nbs1/Xrs2 (MRN/MRX) complex recognizes DSBs and leads to the activation of the damage checkpoint kinase ATM/Tel1 (Lee & Paull, 2004; Lee & Paull, 2005; Usui et al, 2001).

4.2 Checkpoint response to replication stress

In addition to the cell cycle regulation, the activated checkpoint pathways regulate gene expression through modification of transcription factors (Huang et al, 1998), and inhibit the replication initiation from late origins (Santocanale & Diffley, 1998; Shirahige et al, 1998). The inhibition of late origins is mediated by the checkpoint kinase-dependent phosphorylation of two of the essential replication initiators, DDK and Sld3 (Lopez-Mosqueda et al, 2010; Zegerman & Diffley, 2010). This output of the checkpoint response may contribute to the genome integrity by preventing the formation of additional number of stalled forks (Lopez-Mosqueda et al, 2010). The replication checkpoint kinases prevent stalled replication forks from breaking down (Desany et al, 1998; Lopes et al, 2001; Tercero & Diffley, 2001). In fission yeast, the Rad3 kinase is activated in response to replication stress, and it phosphorylates the downstream Cds1 kinase, which in turn phosphorylates various downstream target proteins including the structure-specific nuclease Mus81 (Boddy et al, 2001; Kai et al, 2005). The phosphorylation of Mus81 by the Cds1 kinase results in dissociation of Mus81 from chromatin, preventing it from cleaving the stalled fork. Another example of the checkpoint target is Rad60, which is required for recombinational repair probably through the regulation of the Smc5/6 complex (Miyabe et al, 2006; Morishita et al, 2002). The checkpoint kinase phosphorylates Rad60, leading to its delocalization from the nucleus after replication stress (Boddy et al, 2003; Miyabe et al, 2009). These observations are consistent to the idea that the replication checkpoint prevents recombinational repair to occur as long as the stalled forks are able to keep the checkpoint activated. Intriguingly, the

MCM proteins are phosphorylated in response to replication stress through the checkpoint kinases (Bailis et al, 2008; Cortez et al, 2004b; Ishimi et al, 2003; Randell et al, 2010; Yoo et al, 2004b). The phosphorylation of the MCM proteins may increase the stability of the complex in the stalled condition (Randell et al, 2010).

5. Regulation of the replication initiation to maintain the genome integrity

Many pre-RCs are assembled on each chromosome in late M to G1 phases, but replication initiates recurrently from only a subset of them leaving the rest of them dormant. However, when replication forks are collapsed, replication initiates from the nearby dormant origins (Doksani et al, 2009), indicating the importance of the dormant origins under the stressed condition. Even under the normal growth condition, the damage checkpoint operates to stabilize a chromosome when all or almost all of the efficient replication origins on the chromosome are deleted (Theis et al, 2010). It is likely that during the long travel on DNA, replication forks spontaneously collapse to create DSBs. Consistent to this idea, decreasing the replication initiation by partial inhibition of the assembly of pre-RCs causes gross chromosomal rearrangements (GCRs) (Tanaka & Diffley, 2002). The most common fragile site in human lymphocytes FRA3B is caused by a paucity of replication initiation events in that region (Letessier et al, 2011). A hypomorphic mutation of Mcm4^{Chaos3}, that decreases the Mcm2-7 protein levels, in mice causes various types of chromosome instability and shows predisposition to cancer (Chuang et al, 2010; Kawabata et al, 2011; Shima et al, 2007). These findings demonstrate that the number and the distribution of replication initiation along a chromosome are important for maintaining the genome stability.

Polo-like kinase (PLK) is involved in various important cellular events such as regulation of mitotic entry, chromosome segregation, centrosome maturation, and mitotic exit. PLK contains two tandem Polo boxes, termed as the Polo-box domain that interacts with phosphoproteins (Elia et al, 2003). The Xenopus PLK homolog Plx1 binds to Claspin in a manner dependent on the phosphorylation of Claspin by the checkpoint kinase (Yoo et al, 2004a). The Plx1 phosphorylates Claspin and causes its dissociation from chromatin, resulting in the inactivation of the replication checkpoint kinase Chk1 after a prolonged checkpoint arrest. The PLK homolog in budding yeast Cdc5 is also required for the down regulation of the replication checkpoint: the adaptation, that is the resumption of the cell cycle in the presence of a single unrepaired DSB after a prolonged arrest (Donnianni et al, 2010; Toczyski et al, 1997; Vidanes et al, 2010). These findings indicate that PLK has an inhibitory effect on the checkpoint response. Members of the MCM helicase are phosphorylated by the checkpoint kinases, suggesting a regulation of the MCM helicase by the ATR/ATM checkpoint (Cortez et al, 2004a; Yoo et al, 2004b). Mammalian PLK binds to the Mcm proteins in the phosphorylation dependent manner (Lowery et al, 2007; Tsvetkov & Stern, 2005). In Xenopus, the phosphorylation of Mcm2 by ATR stimulates the interaction with Plx1, probably recruiting Plx1 to the damage sites (Trenz et al, 2008). Although Plx1 is dispensable for DNA replication under the normal condition, it is essential to complete DNA replication when there are only a limited number of pre-RCs assembled on chromatin or in the presence of a low dose of replication inhibitors such as aphidicolin. Thus, it seems that, even though the overall level of the initiation of DNA replication is prohibited in the presence of replication problem, the inhibitory effect caused by the checkpoint is relieved by the Polo-like kinaseso that replication initiation occurs from the dormant origins nearby the stalled or collapsed fork.

6. Coupling DNA unwinding with DNA synthesis under the stressed condition

In bacteria, the replication fork DNA helicase (DnaB) and the replicative DNA polymerase (Pol III holoenzyme) are associated with each other, and the interaction is essential for a high rate of replication fork movement of about 1,000 nt/s (Kim et al, 1996). In eukaryotes, the CMG complex unwinds template DNA strands and specific DNA polymerases (Pol α, δ and ε) synthesize their respective leading and lagging strands. When the polymerase function is interfered by replication stress, the CMG complex continues to unwind dsDNA to some extent, and induce the activation of the replication checkpoint. However, unregulated continuous translocation of the CMG helicase exposes longer stretch of ssDNA (Figure 2). The uncoupling of the helicase from the polymerase may cause re-annealing of the two complementary single-strands behind the helicase, which is a serious obstacle at the time of resumption of DNA synthesis. In addition, the long ssDNA is likely to be fragile compared with the short one.

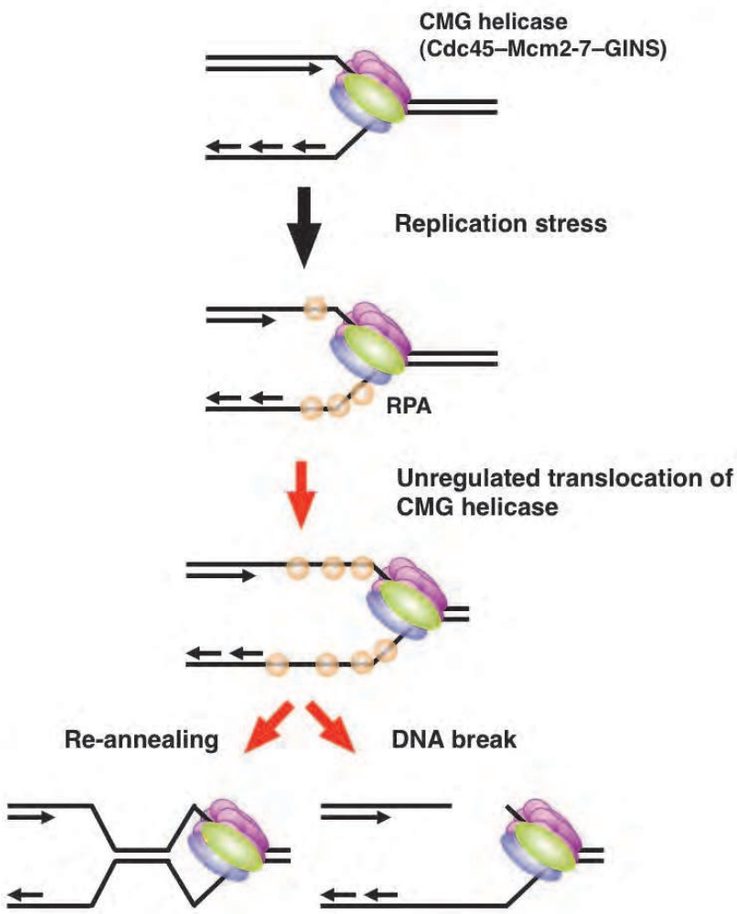

Fig. 2. Unregulated translocation of CMG helicase disrupts the replication fork.

No direct functional interaction between DNA polymerases and the CMG complexes has been reported, so far. However, in budding yeast, elimination of Tof1, Csm3 or Mrc1 causes uncoupling of the CMG helicase from DNA synthesis (Katou et al, 2003). Mrc1 may bridge

the gap between the leading strand polymerase and the CMG helicase, as Mrc1 interacts with both DNA pol ε (Lou et al, 2008) and the MCM protein (Bando et al, 2009; Komata et al, 2009). On the other hand, Ctf4 may bridge the gap between the lagging strand polymerase and the CMG helicase as Ctf4 interacts with DNA Pol α (Miles & Formosa, 1992) and with MCM and GINS (Gambus et al, 2009). The connection between the polymerase and helicase mediated by Mrc1 and Ctf4 may be important to couple the two important reactions of replication (Figure 3A). When DNA synthesis is inhibited, higher-order conformation around DNA polymerases would change. The conformational change emanates a signal that may be transmitted via the bridge molecules to the CMG helicase, to prevent uncoupling of the unwinding from the synthesis. Consistent to this model, mutations in the CMG complex suppress the hypersensitivity of fission yeast *mrc1* cells to HU (Nitani et al, 2006).

Another model is also proposed, in which the checkpoint kinase controls the activity of the CMG helicase (Figure 3B). The MCM proteins are phosphorylated dependent on the replication checkpoint kinases (Bailis et al, 2008; Cortez et al, 2004b; Ishimi et al, 2003; Randell et al, 2010; Yoo et al, 2004b). As phosphorylation of MCM down regulates the DNA unwinding activity of Mcm4-6-7 (Ishimi et al, 2003), it is possible that once the replication checkpoint kinase becomes activated with the aid of the CMG helicase, it turns off the activity of CMG.

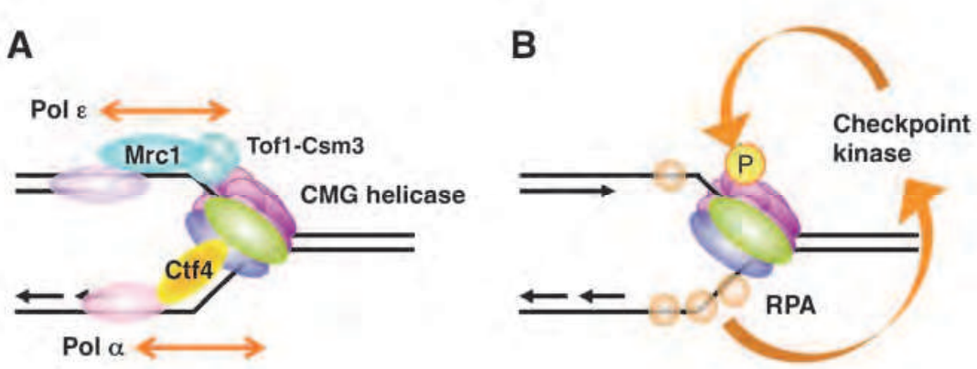

Fig. 3. Down regulation of the CMG helicase in response to replication stress.

The evolutionally conserved C-terminal domain (CTD) of Mcm4 plays an important role in the regulation of DNA unwinding carried out by the MCM helicase, as the deletion of the C-terminal in fission yeast accumulates RPA on the stalled replication forks while binding of MCM and GINS remains unaffected (Nitani et al, 2008). A recent study has shown that among Mcm2-7 subunits Mcm4 has the strongest affinity to both GINS and Cdc45 (Ilves et al, 2010). Thus, it is possible that the C-terminal region of Mmc4 affects the configuration of the CMG complex thereby regulating its helicase activity when DNA synthesis is inhibited. It is important to understand the interaction between the components of CMG, as is modulated in response to replication stress.

There is increasing body of evidence that regulation of the CMG helicase is important not only for DNA replication but also for maintaining the genome integrity. Recent findings that hypomorphic mutations in the replication proteins such as MCM in mammals cause growth abnormality and or predisposition to cancer, underscore the importance of the tight regulation of DNA replication. Genome-wide analysis of replication origins in different

types of tissues or cells would reveal the specificity of cancer to certain types of tissues. Detailed studies of spatiotemporal regulation of the integral component of the replication fork, the CMG helicase, would provide great insights into the mechanism by which chromosomal DNA is faithfully replicated in the S phase, which is one of the essential events during cell proliferation.

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8. References

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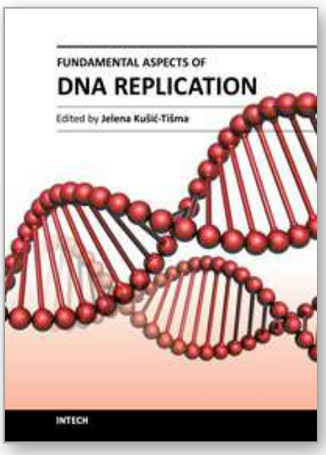

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DNA replication, the process of copying one double stranded DNA molecule to produce two identical copies, is at the heart of cell proliferation. This book highlights new insights into the replication process in eukaryotes, from the assembly of pre-replication complex and features of DNA replication origins, through polymerization mechanisms, to propagation of epigenetic states. It also covers cell cycle control of replication initiation and includes the latest on mechanisms of replication in prokaryotes. The association between genome replication and transcription is also addressed. We hope that readers will find this book interesting, helpful and inspiring.

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