

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Cell Cycle Regulation of DNA Replication in *S. cerevisiae*

Jorrit M. Enserink

Centre for Molecular Biology and Neuroscience (CMBN)

Oslo University Hospital, Oslo

Norway

## 1. Introduction

Cell duplication is a strictly regulated process that underlies growth and development of all organisms. The ordered series of events that lead to the duplication of a cell is commonly referred to as the cell cycle. The purpose of the cell cycle is to transmit an intact and complete copy of the genome from one generation to the next. Although certain highly specialized cell types undergo multiple rounds of replication per cell cycle in a developmentally coordinated process termed endoreplication, like for example megakaryocytes, plant endosperm, *Drosophila* follicle and nurse cells, and rodent trophoblasts (Lee, Davidson et al. 2009), the vast majority of cells in an organism replicate their DNA only once per cell cycle. Endoreplication differs from the aberrant process of re-replication in that it is highly regulated, and DNA content increases by clearly delineated genome doublings, whereas re-replication results from unscheduled activation of the DNA replication process (Lee, Davidson et al. 2009). Re-replication can result in genome instability that can have adverse effects on the well-being of the organism. Multiple mechanisms have evolved that prevent re-replication by restricting DNA replication to one specific phase of the cell cycle. Several protein complexes required for the various steps of DNA replication are separated in time and space during the cell cycle, and are only active during brief phases of the cell cycle. In this chapter I will discuss regulation of DNA replication by the cell cycle.

## 2. The cell cycle

### 2.1 Regulation of the cell cycle by cyclin dependent kinases

The cell cycle is generally divided into four specific phases: G1 phase, during which the cell grows and prepares for cell cycle entry; S phase, during which DNA synthesis takes place; G2 phase, during which cells prepare for M phase; and M phase, in which chromosomes segregate and cells divide. In eukaryotic cells, the cell cycle is controlled by cyclin dependent kinases (CDKs; Figure 1). CDKs have been highly conserved during evolution; even to such a degree that vertebrate CDK1 can substitute for *Saccharomyces cerevisiae* Cdk1. A single CDK, Cdk1 (also known as Cdc28) is necessary and sufficient for cell cycle control in *S. cerevisiae* (Enserink and Kolodner 2010). Nine different cyclins form complexes with Cdk1 throughout the cell cycle to regulate efficient cell cycle progression. In higher

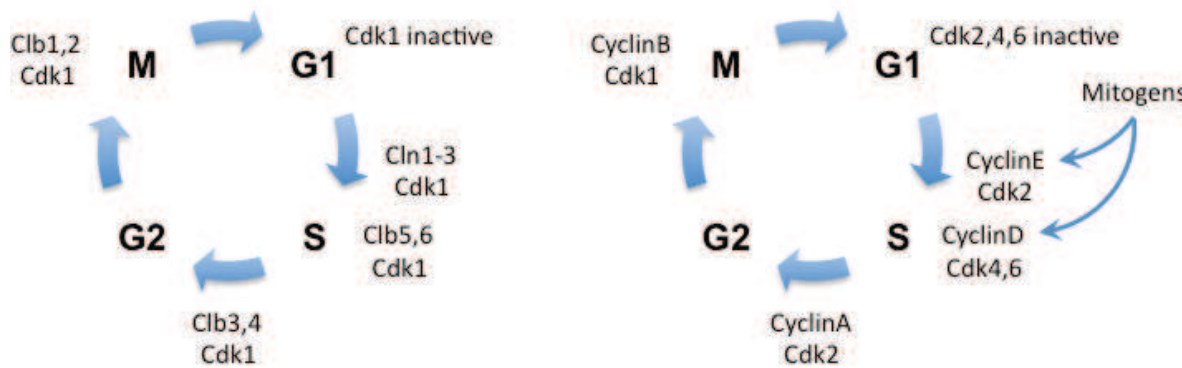


Fig. 1. Regulation of the cell cycle by CDKs. Cell cycle regulation by CDKs is highly conserved throughout evolution. A single CDK regulates the cell cycle in *S. cerevisiae*, while multiple CDKs have been associated with cell cycle control in higher eukaryotes. Nonetheless, a single CDK suffices for cell cycle control in higher eukaryotes, except during embryogenesis.

eukaryotes, several CDKs are involved in cell cycle regulation, i.e. Cdk1, Cdk2, Cdk4 and Cdk6 (Satyanarayana and Kaldis 2009). Mitogens induce transcription of cyclin D and cyclin E, which form complexes with Cdk4/6 and Cdk2, respectively, to induce entry into the cell cycle. Cdk1 is traditionally thought of as a mitotic CDK that is only involved in regulation of mitosis (Satyanarayana and Kaldis 2009). However, mouse knockout models have changed this paradigm; only Cdk1 was shown to be necessary and sufficient for cell cycle control, while Cdk2/4/6 do not suffice to regulate the cell cycle (Malumbres and Barbacid 2009). Instead, Cdk2/4/6 are important for development of the organism by controlling the cell cycle in cells of specialized tissues (Malumbres and Barbacid 2009). Note that this single-CDK model is very similar to the yeast cell cycle model in which a single CDK suffices for cell cycle regulation.

## 2.2 Regulation of cyclin dependent kinases

CDK activity is controlled at multiple levels (Figure 2). CDKs are inactive during G1 phase because cyclin levels are low (Pines 1994). As the cell progresses through G1 phase, cyclin levels increase, and binding of cyclin to CDK increases the catalytic activity of the kinase (Jeffrey, Russo et al. 1995). CDKs, like other kinases, have a two-lobed structure. In absence of cyclins the catalytic cleft is blocked by a large, flexible structure called the T loop, and the phosphates of the ATP molecule are improperly aligned (Pavletich 1999). When cyclins bind, the T loop moves away from the catalytic cleft, and the phosphates of the ATP molecule realign, allowing phosphorylation of substrate proteins. In addition, phosphorylation of the T loop increases the affinity of the CDK for cyclins and further exposes the catalytic cleft (Russo, Jeffrey et al. 1996). CDK activity is negatively regulated by cyclin dependent kinase inhibitors (CKIs), such as p21<sup>WAF</sup>, p27<sup>CIP</sup> and p16<sup>INK4</sup>. CKIs directly bind cyclin-CDK complexes, and inhibit binding of ATP to the kinase (p27<sup>CIP</sup>) or prevent binding of the cyclin (p16<sup>INK4</sup>) (Pavletich 1999). Furthermore, CDKs can be phosphorylated on residues in the N-terminus by Wee1 kinases, leading to inhibition of CDK activity. For instance, *S. cerevisiae* Cdk1 is phosphorylated on Y19 by Swe1, which prevents entry into M phase (Booher, Deshaies et al. 1993). Phosphatases of the Cdc25 family (Mih1 in *S. cerevisiae*) dephosphorylate these N-terminal residues to alleviate inhibition of Cdk1, thereby allowing the cell to resume the cell cycle (Russell and Nurse 1986).

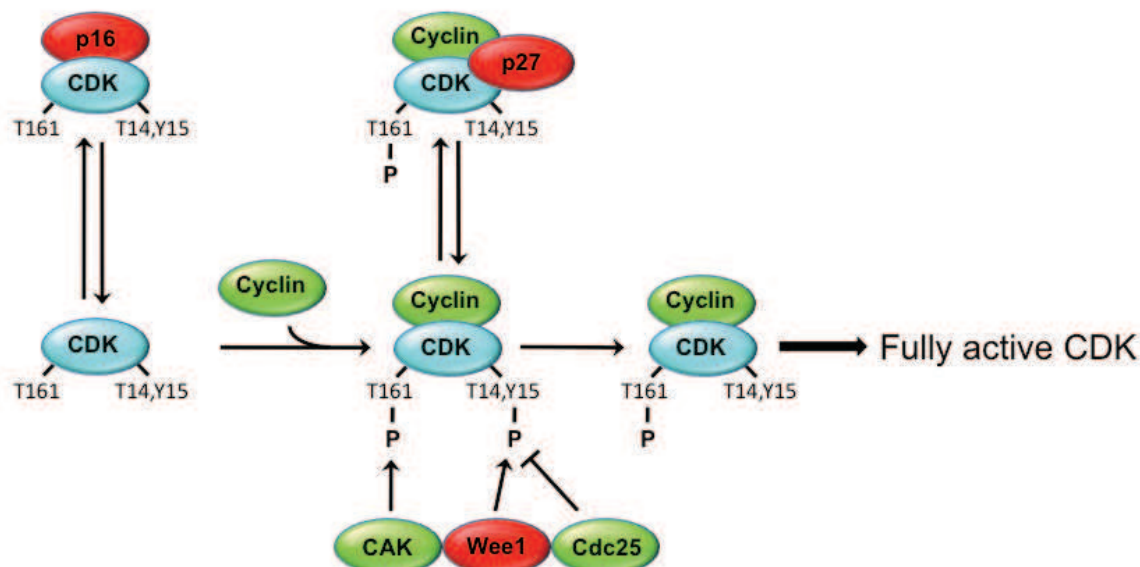


Fig. 2. Regulation of CDK activity. CAK is composed of three subunits in higher eukaryotes and consists of Cdk7, cyclin H and MAT1, while in *S. cerevisiae* the kinase Cak1 provides CAK activity. T161 is similar to T163 in *S. cerevisiae*, and T14 and Y15 correspond to *S. cerevisiae* Y19. See text for details.

### 2.3 Positive feedback induces entry into S phase

A notable feature of cell cycle regulation is the abundant use of feedback loops, which make cell cycle transitions particularly switch-like (Ferrell, Tsai et al. 2011). One example is the transition from G1 to S phase (Fig. 3A). During G1 phase, CDKs are inactive due to low cyclin levels and the presence of CKIs. However, as the cell progresses through G1, cyclin levels (Cln3 in *S. cerevisiae*) gradually increase. Cln3-Cdk1 complexes then phosphorylate a protein called Whi5 (Costanzo, Nishikawa et al. 2004; de Bruin, McDonald et al. 2004). Whi5 is a transcriptional suppressor that interacts with SBF, a transcription factor complex required for transcription of genes involved in cell cycle entry and DNA replication (the so-called G1 transcriptional program). Whi5 also recruits histone deacetylases (HDACs) that maintain the chromatin surrounding the SBF-Whi5 complex in a repressive state (Huang, Kaluarachchi et al. 2009; Wang, Carey et al. 2009). Thus, as long as Whi5 is bound to SBF, the cell cannot enter the cell cycle. However, phosphorylation of Whi5 by Cln3-Cdk1 induces the release of Whi5 and HDACs from SBF (Costanzo, Nishikawa et al. 2004; de Bruin, McDonald et al. 2004; Huang, Kaluarachchi et al. 2009; Wang, Carey et al. 2009). SBF then activates transcription of the G1 transcriptional program. Importantly, several cyclins (e.g. Cln1 and Cln2) are part of this transcriptional program, and in a positive feedback loop the newly synthesized cyclins will further activate Cdk1 to phosphorylate more Whi5, leading to further activation of SBF (Charvin, Oikonomou et al. 2010). This positive feedback loop makes cell cycle entry behave like a switch, ensuring coherent cell cycle entry. Cell cycle entry also requires destruction of the CKI Sic1, which inhibits Clb5-Cdk1 and Clb6-Cdk1 (Schwob, Bohm et al. 1994). Clb5,6-Cdk1, as we will see below, activate DNA replication. Sic1 is phosphorylated by Cln-Cdk1 complexes, which targets it for destruction by the SCF (Nash, Tang et al. 2001). Thus, both Whi5 and Sic1 need to be inhibited for efficient cell cycle entry.

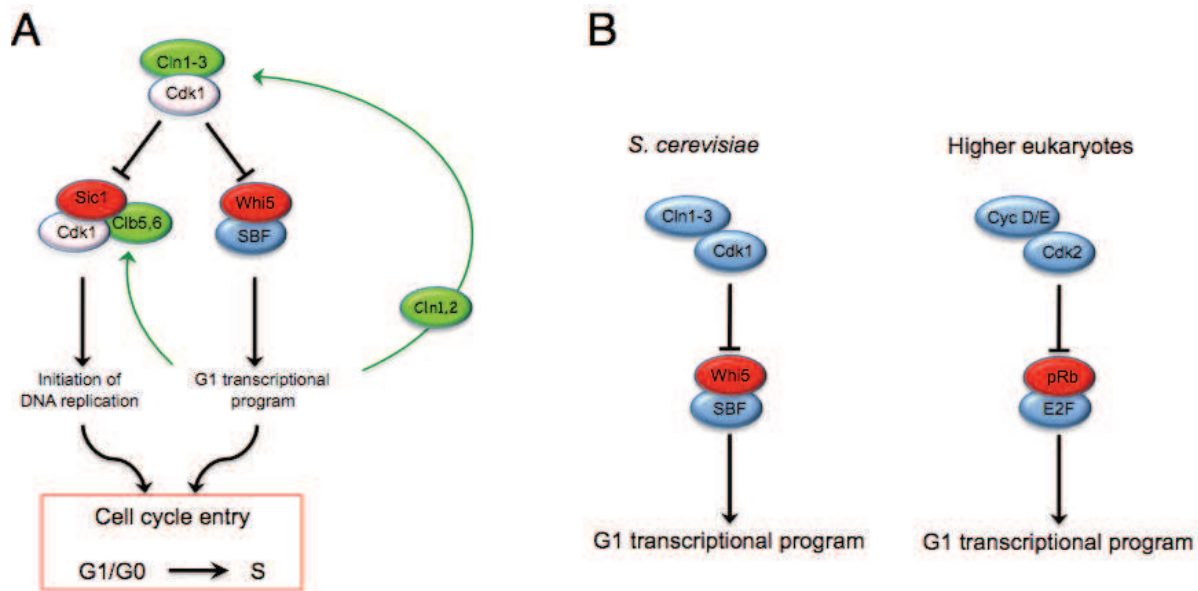


Fig. 3. Regulation of cell cycle entry in *S. cerevisiae*. A, Efficient cell cycle entry requires feedback mechanisms. Cln3-Cdk1 initially phosphorylates a small amount of Whi5, leading to minor activation of the G1 transcriptional program. Cyclins *CLN1*, *CLN2*, *CLB5* and *CLB6* are part of this program, and in a positive feedback loop, Cln1 and Cln2 will bind and activate Cdk1, leading to phosphorylation of more Whi5. DNA replication will not start before the CKI Sic1, which inhibits Clb5-Cdk1 and Clb6-Cdk1 complexes, is degraded. Sic1 degradation is initiated by phosphorylation by Cln1,2-Cdk1 complexes, resulting in degradation of Sic1 by the SCF. B, The mechanism of cell cycle control is highly conserved between eukaryotes.

The mechanism of cell cycle entry is highly conserved between *S. cerevisiae* and higher eukaryotes (Fig. 3B). For instance, in vertebrates the retinoblastoma protein, pRB, binds and thereby inhibits the transcription factor E2F. pRB also recruits HDAC complexes to keep the surrounding chromatin in a repressed state. Upon phosphorylation by cyclin D-Cdk4,6 and cyclin E-Cdk2 complexes, pRB and the HDACs dissociate from E2F (van den Heuvel and Dyson 2008). E2F then activates a transcriptional program that mediates expression of proteins involved in cell cycle entry and DNA replication.

Once the cell has successfully entered the cell cycle, it shuts off the G1 transcriptional program. This is important for proper cell cycle progression and promotes unidirectionality of the cell cycle. In *S. cerevisiae*, one important mechanism of shutting off the G1 transcriptional program involves the transcriptional repressor Nrm1 (de Bruin, Kalashnikova et al. 2006). Nrm1 inhibits transcription of the G1 transcriptional program. In addition, the G1 cyclins Cln1 and Cln2 are degraded, leading to dephosphorylation of Whi5, thereby allowing it to re-enter the nucleus to inhibit the G1 transcriptional program. Finally, the G1 transcriptional program is inhibited by Clb-Cdk1 complexes, which phosphorylate and inhibit the SBF transcription factor complex (Koch, Schleiffer et al. 1996).

### 3. CDKs control DNA replication at multiple levels

The purpose of the cell cycle is to faithfully transmit a complete copy of the genome from one generation to the next. Two events are fundamental to this process: DNA replication,

and segregation of the replicated chromosomes into the daughter cells. It is important that chromosome segregation does not occur before DNA replication is complete, otherwise the cell would experience massive genome instability. Therefore, these two processes are separated in time; chromosome segregation (which takes place in M phase) does not occur until DNA replication (which occurs in S phase) has finished (Murray and Kirschner 1989). Furthermore, as I will discuss below, several mechanisms have evolved that make sure that DNA replication only takes place during S phase and just once per cell cycle.

### 3.1 Licensing of origins of replication is restricted to G1 phase

DNA replication is a multi-step process. It starts with origin licensing, a process in which a specific protein-DNA complex called the prereplicative complex (pre-RC) is formed at origins in G1 phase, when Cdk1 is inactive (Fig. 4 and Fig. 5). In *S. cerevisiae*, origins were first identified by their capability to sustain replication of mini-chromosomes and plasmids, and therefore these sequences are commonly referred to as Autonomously Replicating Sequences (ARSs) (Stinchcomb, Struhl et al. 1979). ARSs typically are 100-200 bp in length with little sequence conservation, except for an 11 bp ARS consensus sequence (5'-WTTTAYRTTTW-3'), and mutations in this sequence abolish the function of the ARS (Theis and Newlon 1997; Newlon and Theis 2002). The ARS consensus sequence is recognized by the Origin of Replication Complex (ORC), which consists of Orc1, Orc2, Orc3, Orc4, Orc5 and Orc6 (Bell and Stillman 1992). The ORC is constitutively associated with the ARS throughout the cell cycle. The ORC recruits the ATPase Cdc6, the DNA replication licensing factor Cdt1 and the Mcm2-7 helicase complex (Araki 2010). Loading of the Mcm2-7 complex is what defines pre-RC formation; it confers DNA replication competence to the cell and is the first regulated step of DNA replication before actual DNA synthesis. At this stage, the Mcm2-7 helicase complex is associated with the ORC but still inactive; pre-RC formation is followed by initiation of DNA replication, during which Mcm2-7 is activated.

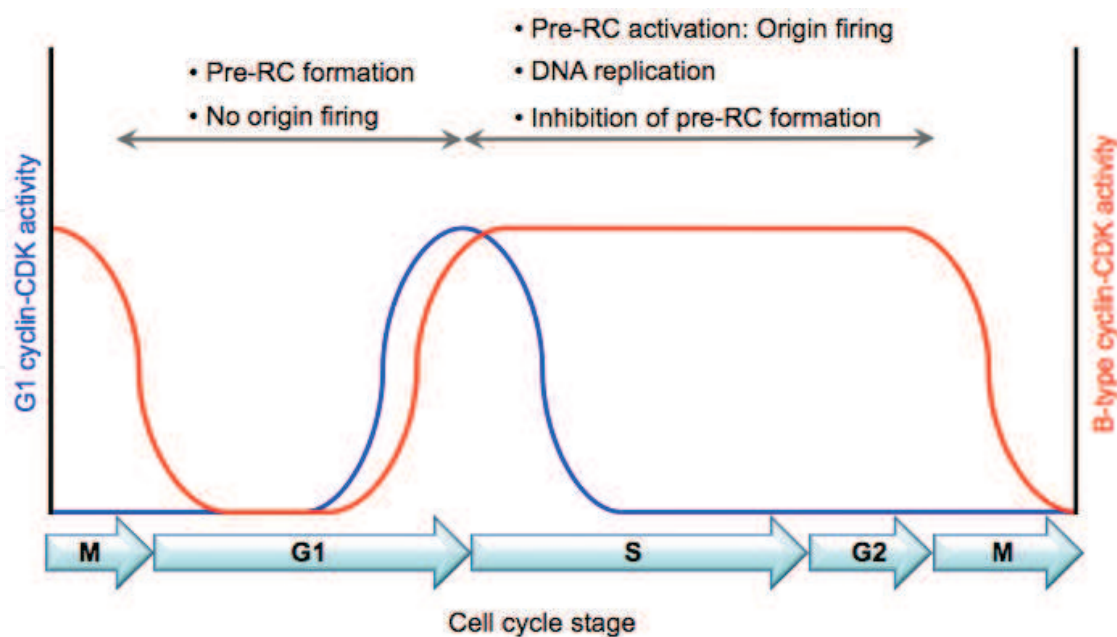


Fig. 4. Licensing of origins of replication can only take place in G1 phase when Cdk1 is inactive, while origin activation only occurs during S phase when Cdk1 is active. See text for details.

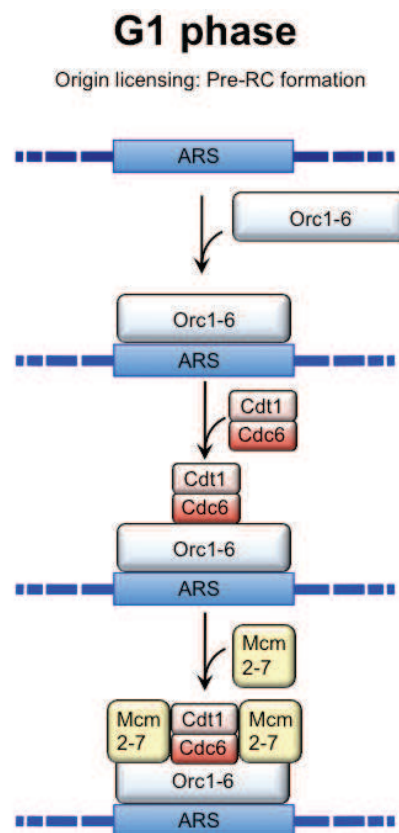


Fig. 5. Regulation of DNA replication by the cell cycle: Origin licensing. Origin licensing takes place during G1 phase, when Cdk1 activity is low. The ORC becomes associated with ARS DNA directly after DNA replication and stays associated with DNA throughout the cell cycle. The ORC serves as a platform for Cdt1 and Cdc6 recruitment to the origin. Finally, an origin is replication competent when the MCM2-7 complex arrives.

### 3.2 Phosphorylation of DNA replication factors induces firing of origins of replication during S phase

Clb5-Cdk1 and Clb6-Cdk1 complexes are responsible for inducing DNA replication (Schwob and Nasmyth 1993). The activity of these complexes is restricted to S phase because the Clb5,6 cyclin genes are part of the G1 transcriptional program, and therefore expression of Clb5,6 peaks at the G1-S transition. However, Clb5,6-Cdk1 complexes are still maintained in an inactive state by the CKI Sic1 until the cell is ready to initiate DNA replication (Schwob, Bohm et al. 1994). Cln1,2-Cdk1 complexes then phosphorylate Sic1, which leads to ubiquitination of Sic1 by the Skp1-Cullin-F-box (SCF) complex, targeting it for degradation by the proteasome (Nash, Tang et al. 2001). This then results in activation of Clb5,6-Cdk1 complexes.

Clb5,6-Cdk1 complexes induce DNA replication by phosphorylating Sld2 and Sld3 (Fig. 6) (Araki 2010). Phosphorylation of Sld2 and Sld3 increases their affinity for Dpb11 (Araki 2010). Dpb11 has two pairs of tandem BRCT domains, which are known to be phosphopeptide binding domains; the N-terminal pair of BRCT domains interact with Sld3, while the C-terminal pair preferentially bind Sld2 (Araki 2010). The interaction between Dpb11, Sld2 and Sld3 is necessary and sufficient for initiation of DNA replication, although the exact molecular mechanism remains obscure (Araki 2010).

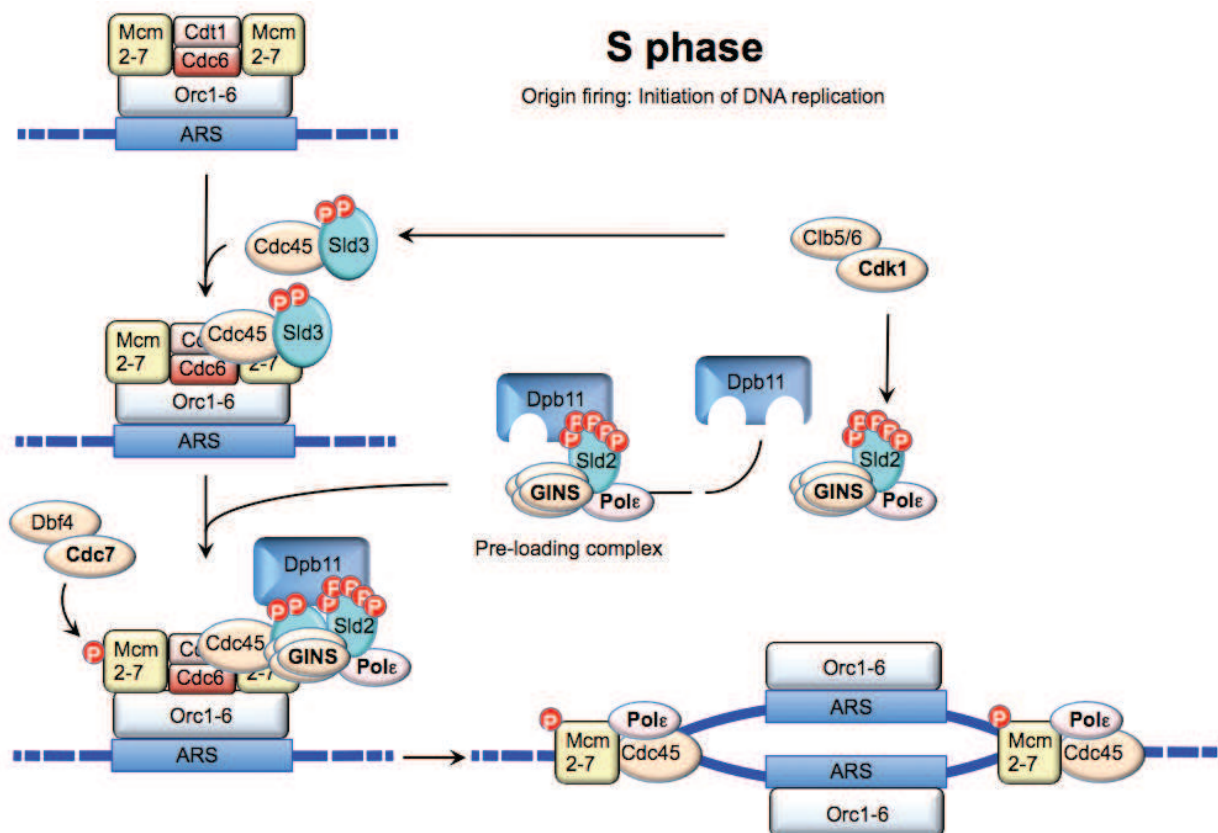


Fig. 6. Regulation of DNA replication by the cell cycle: Origin firing. At the end of G1 phase, cyclin-Cdk1 complexes phosphorylate Sld2 and Sld3, leading to recruitment of GINS and Pol $\epsilon$  to the origin. The additional phosphorylation of MCM2-7 by DDK results in formation of a complex between Cdc45, GINS and MCM2-7, which then induces unwinding of DNA and initiation of DNA replication.

It was recently reported that phosphorylation of Sld2 may promote formation of the pre-loading complex (pre-LC), which consists of Sld2, Dpb11, GINS and Pol $\epsilon$  (Muramatsu, Hirai et al. 2010). GINS and Pol $\epsilon$  form a subcomplex throughout the cell cycle, which associates with Sld2-Dpb11 upon phosphorylation of Sld2 by Cdk1 (Muramatsu, Hirai et al. 2010). One proposed function for formation of the pre-LC is that the pre-LC serves to bring the GINS complex to the pre-RC complex through the interaction of Dpb11 with the pre-RC-associated, Cdk1-phosphorylated Sld3 (Araki 2010). Arrival of GINS at the pre-RC would then result in formation of the Cdc45-MCM2-7-GINS (CMG) complex, which represents the active helicase that unwinds DNA to mediate DNA replication (Araki 2010).

In addition to Clb5,6-Cdk1, a second kinase complex termed Dbf4-dependent kinase (DDK), which consists of the regulatory subunit Dbf4 and the catalytic subunit Cdc7, controls initiation of DNA replication (Araki 2010). DDK is only active during S phase, because Dbf4 is only expressed in late G1, and because Dbf4 is degraded by the APC (Tanaka and Araki 2010). Because Cdk1 phosphorylates and inactivates components of the APC (Jaspersen, Charles et al. 1999; Ostapenko, Burton et al. 2008), Dbf4 is stabilized when Cdk1 becomes active, i.e. at the G1/S transition (Tanaka and Araki 2010). DDK is recruited to origins independently of the pre-LC complex, probably through its interaction with the MCM2-7. DDK directly phosphorylates the MCM2-7 complex, which is thought to increase its affinity



for Cdc45 and GINS, thus leading to formation of the CMG complex (Tanaka and Araki 2010). DNA pol $\epsilon$  extends the leading strand at each replication fork, while DNA Pol $\alpha$ /primase synthesizes Okazaki fragments that are extended by DNA Pol $\delta$  on the lagging strand.

The requirement for Cdk1 can be bypassed to induce DNA replication outside of S phase. To circumvent the requirement for Cdk1, both Sld2 and Sld3 phosphorylation must be bypassed (Araki 2010). The requirement for Sld2 phosphorylation can be bypassed by expressing a phospho-mimetic form of Sld2, either Sld2-T84D or Sld2-11D. The requirement for Sld3 can be bypassed by expressing an Sld3-Dpb11 fusion protein. However, cells that express phospho-mimetic Sld2 and Sld3-Dpb11 still depend on DDK1 activity to induce DNA replication (Araki 2010). Dbf4 is normally unstable in G1 phase, but overexpressing Dbf4 in combination with expressing Sld2-T84D/Sld2-11D and Sld3-Dpb11 leads to DNA replication in G1 phase.

### 3.3 Expression of most DNA replication factors is limited to a specific phase of the cell cycle

Importantly, the expression of many genes that encode DNA replication factors is confined to a specific phase of the cell cycle (Table 1). In general, genes that have functions in early stages of the DNA replication process (i.e. licensing of origins of replication) are transcribed in G2 and M phase, such that their protein levels are highest in G1 phase. Genes that function later in the DNA replication process (e.g. proteins that are part of the DNA replication complex) peak during late G1, such that their levels are highest in S phase when DNA replication takes place. The confined expression of DNA replication factors to specific phases of the cell cycle plays an important role in restricting DNA replication to S phase (Enserink and Kolodner 2010).

Gene	Description	Maximum expression	Function in DNA replication
<i>ORC3</i>	Subunit of the origin recognition complex	Late S	Pre-RC complex
<i>ORC1</i>	Subunit of the origin recognition complex	G2	Pre-RC complex
<i>ORC4</i>	Subunit of the origin recognition complex	G2	Pre-RC complex
<i>ORC6</i>	Subunit of the origin recognition complex	G2	Pre-RC complex
<i>CDT1</i>	DNA replication licensing factor	G2	Pre-RC complex
<i>MCM2</i>	Helicase	Late M	Replicative helicase
<i>MCM3</i>	Helicase	Late M	Replicative helicase
<i>MCM4</i>	Helicase	Late M	Replicative helicase
<i>MCM5</i>	Helicase	Late M	Replicative helicase
<i>MCM6</i>	Helicase	Late M	Replicative helicase
<i>MCM7</i>	Helicase	Late M	Replicative helicase
<i>CDC6</i>	Component of the pre-RC complex	Late M	Pre-RC complex
<i>SLD2</i>	Required for initiation of DNA replication	Late G1	Origin firing
<i>CLB5</i>	Cyclin involved in Sld2 phosphorylation	Late G1	Origin firing
<i>CLB6</i>	Cyclin involved in Sld2 phosphorylation	Late G1	Origin firing
<i>CSM3</i>	Required for stable replication fork pausing	Late G1	Replication fork

Gene	Description	Maximum expression	Function in DNA replication
<i>TOF1</i>	Required for stable replication fork pausing	Late G1	Replication fork
<i>PSF1</i>	GIN5 complex	Late G1	Replicative helicase
<i>PSF2</i>	GIN5 complex	Late G1	Replicative helicase
<i>DBF4</i>	Regulatory subunit of Cdc7-Dbf4 kinase complex	Late G1	Origin firing
<i>DPB11</i>	Loads DNA pol $\epsilon$ onto pre-RCs	Late G1	Origin firing
<i>RFA1</i>	Replication factor A complex	Late G1	Replication fork
<i>RFA2</i>	Replication factor A complex	Late G1	Replication fork
<i>RFA3</i>	Replication factor A complex	Early S	Replication fork
<i>POL30</i>	PCNA	Late G1	Replication fork
<i>CDC45</i>	DNA replication initiation factor; recruited to MCM pre-RC complexes	Late G1	Replicative helicase
<i>MRC1</i>	DNA replication and checkpoint signaling	Late G1	Replication fork
<i>POL2</i>	DNA polymerase $\epsilon$ catalytic subunit	Late G1	Replication fork
<i>POL3</i>	DNA polymerase $\delta$ catalytic subunit	Late G1	Replication fork
<i>POL31</i>	DNA polymerase $\delta$ subunit	Late G1	Replication fork
<i>POL32</i>	DNA polymerase $\delta$ subunit	Late G1	Replication fork
<i>POL12</i>	DNA polymerase $\alpha$ primase subunit	Late G1	Replication fork
<i>CDC9</i>	Ligase that joins Okazaki fragments	Late G1	Replication fork
<i>RNH201</i>	RNase H2, degrades Okazaki fragments	Late G1	Replication fork
<i>RNH202</i>	RNase H2, degrades Okazaki fragments	Late G1	Replication fork
<i>RAD27</i>	Flap Endonuclease	Late G1	Replication fork
<i>ELG1</i>	Alternative RFC complex component	Late G1	Replication fork
<i>RNR1</i>	Ribonucleotide reductase, dNTP synthesis	Late G1	dNTP synthesis
<i>RFC3</i>	Replication factor C complex	Late G1	PCNA loading
<i>RFC4</i>	Replication factor C complex	Late G1	PCNA loading
<i>RFC5</i>	Replication factor C complex	Late G1	PCNA loading
<i>SLD5</i>	GIN5 complex	G1/S border	Replicative helicase
<i>RNR2</i>	Ribonucleotide reductase, dNTP synthesis	G1/S border	dNTP synthesis
<i>RNR3</i>	Ribonucleotide reductase, dNTP synthesis	G1/S border	dNTP synthesis
<i>CDC7</i>	Catalytic subunit of Cdc7-Dbf4 kinase complex	G1/S border	Origin firing
<i>DPB2</i>	DNA polymerase $\epsilon$ subunit	G1	Replication fork
<i>DPB3</i>	DNA polymerase $\epsilon$ subunit	G1	Replication fork
<i>POL1</i>	DNA polymerase $\alpha$ primase catalytic subunit	Late G1	Replication fork

Table 1. Periodic expression of *S. cerevisiae* genes involved in DNA replication. Source: Cyclebase.org (Gauthier, Jensen et al. 2010)

### 3.4 Preventing re-replication

A major challenge to the cell is to make sure that its genetic material does not get copied more than once. Several mechanisms are in place to prevent that firing of origins takes place outside of S phase, and that origins do not fire more than once per cell cycle (Fig. 7). One major mechanism that prevents re-replication focuses on Cdc6 (Remus and Diffley 2009), and Cdc6 is inhibited at three levels. First, transcription of Cdc6 is limited to G1-S phase (Moll, Tebb et al. 1991). Second, phosphorylation of Cdc6 by Cdk1 targets it for degradation by the SCF (Drury, Perkins et al. 1997). Third, any remaining free Cdc6 is 'mopped up' through phosphorylation by Cdk1, which induces the binding of Cdc6 to Clb2-Cdk1, thereby preventing Cdc6 from licensing origins (Mimura, Seki et al. 2004). Although depletion of Cdc6 efficiently inhibits re-firing of origins, additional mechanisms exist to prevent re-replication. For instance, the MCM2-7 complex and the associated Cdt1 are shuttled out of the nucleus in a Cdk1-dependent manner (Labib, Diffley et al. 1999; Nguyen, Co et al. 2000; Liku, Nguyen et al. 2005). Finally, the ORC complex is inhibited through phosphorylation by Cdk1 (Nguyen, Co et al. 2001).

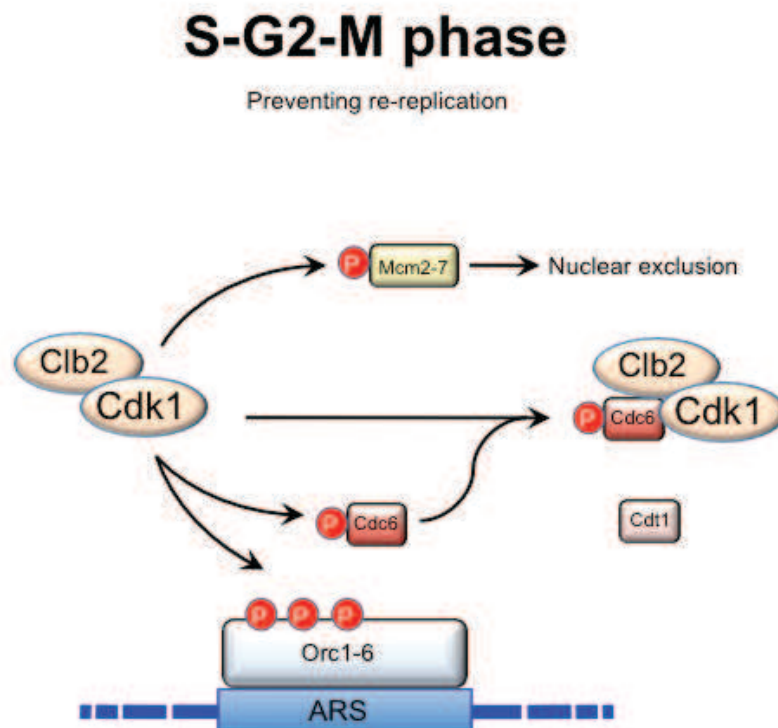


Fig. 7. Mechanisms that prevent re-replication. The Clb2-Cdk1 complex phosphorylates ORC, which prevents ORC from recruiting Cdc6 and Cdt1. Phosphorylation of the Mcm2-7 complex may lead to nuclear export of the helicase complex, as well as the associated Cdt1. Phosphorylation of Cdc6 induces its destruction by the SCF, but also leads to sequestration of Cdc6 by the Clb2-Cdk1, thus preventing Cdc6 from localizing to the ORC.

### 3.5 Precocious activation of Cdk1 leads to DNA damage during S phase

A key feature of tumor cells is uncontrolled entry into the cell cycle, which is nearly always the result of defects in the pRB pathway. Studies in yeast have shown that this is highly detrimental to genome stability (Lengronne and Schwob 2002; Tanaka and Diffley 2002; Enserink, Hombauer et al. 2009). Precocious activation of Cdk1, either through

overexpression of *CLN2-1* (Tanaka and Diffley 2002), encoding a dominant, activated cyclin, or deletion of the CKI *SIC1* (Lengronne and Schwob 2002), leads to unscheduled entry into S phase. The cell now enters S phase when it has not yet finished licensing the origins of replication, and leads to initiation of DNA replication from fewer origins. As a result, the cell experiences increased numbers of DNA double strand breaks, which give rise to genome rearrangements. Interestingly, these adverse effects of precocious S phase entry on genome stability can be suppressed by increasing the number of origins from which cells initiate DNA replication (Lengronne and Schwob 2002; Tanaka and Diffley 2002). Thus, genome instability that results from precocious Cdk1 activation and unscheduled S phase entry is almost entirely the consequence of inefficient activation of DNA replication.

#### 4. Checkpoints monitor DNA replication stress and DNA damage

Stalled replication forks are a threat to the cell's genome because they could collapse, leaving the cell with partially unreplicated, broken chromosomal DNA. Therefore, cells have developed elaborate systems that sense DNA damage and stalling of DNA replication forks to arrest the cell cycle and to induce DNA repair (Fig. 8) (Hartwell and Weinert 1989).

Cells respond to DNA replication blocks and to damaged DNA by activating checkpoints that arrest or slow the cell cycle and then help activate DNA repair pathways (Hartwell and Weinert 1989). The DNA damage checkpoint acts at the G1/S and G2/M borders in the presence of damaged DNA. In addition, two types of checkpoints have been described in S phase: the DNA replication checkpoint, which arrests cell cycle progression and inhibits firing of late replication origins in response to replication stress; and the intra-S checkpoint, which slows DNA replication and cell cycle progression in response to DNA damage (Putnam, Jaehnig et al. 2009). Here I will focus mainly on the DNA replication checkpoint.

Typically, checkpoints are thought to function in a signalling hierarchy: Damage signals → damage sensors → signal transducers → effectors (Fig. 8) (Putnam, Jaehnig et al. 2009). However, several damage sensors are part of the DNA replication fork, and they are themselves targets of these signalling pathways (Putnam, Jaehnig et al. 2009). Therefore, these pathways are not strictly linear.

The DNA replication checkpoint plays a major role in maintenance of genome stability. It is activated when DNA replication forks stall, for instance due to a lesion in the DNA or due to depletion of dNTPs (Branzei and Foiani 2010). Exactly how this is sensed remains unclear, but likely involves exposure of single-stranded DNA (ssDNA) (Byun, Pacek et al. 2005). ssDNA may be generated because the CMG complex keeps unwinding double-stranded DNA, even though it is uncoupled from DNA replication because the replication fork has stalled (Byun, Pacek et al. 2005). The ssDNA binding complex RPA, a heterotrimeric protein complex consisting of Rfa1, Rfa2 and Rfa3, may have a function in sensing ssDNA (Zou and Elledge 2003). Other proteins that have been proposed to act as sensors include Rfc5, PCNA, Dpb11, Pol2 and Sgs1 (Kolodner, Putnam et al. 2002). How these proteins relay the signal to activate the checkpoint is not very clear. Two phosphatidylinositol 3' kinase-related kinases (PIKK) family kinases, Mec1 and Tel1, play a central role in checkpoint activation in *S. cerevisiae* (Kolodner, Putnam et al. 2002). Mec1 is thought to be activated primarily by stalled replication forks, while Tel1 typically responds to damaged DNA (Kolodner, Putnam et al. 2002). As mentioned above, ssDNA is generated upon stalling of replication forks, which is bound by RPA. RPA may recruit the kinase Mec1 and its binding partner Ddc2, leading to activation of Ddc2-Mec1. Mrc1, which is part of the replication fork, becomes

phosphorylated by Mec1, which leads to recruitment of Rad53 (Alcasabas, Osborn et al. 2001). Rad53 is phosphorylated by Mec1, but also by itself, leading to its activation. Because Mrc1 mediates the signal to downstream kinases it is often referred to as a mediator. Another mediator is Rad9, which also activates Rad53 (Gilbert, Green et al. 2001) but is thought to function mainly downstream of Tel1.

In addition to Rad53, another kinase, Chk1, becomes activated by the checkpoint, while the kinase Dun1 is thought to function downstream of Rad53 (Putnam, Jaehnig et al. 2009). Together, these kinases phosphorylate a large number of substrates involved in cell cycle control, DNA repair and nucleotide metabolism (Fig. 8).

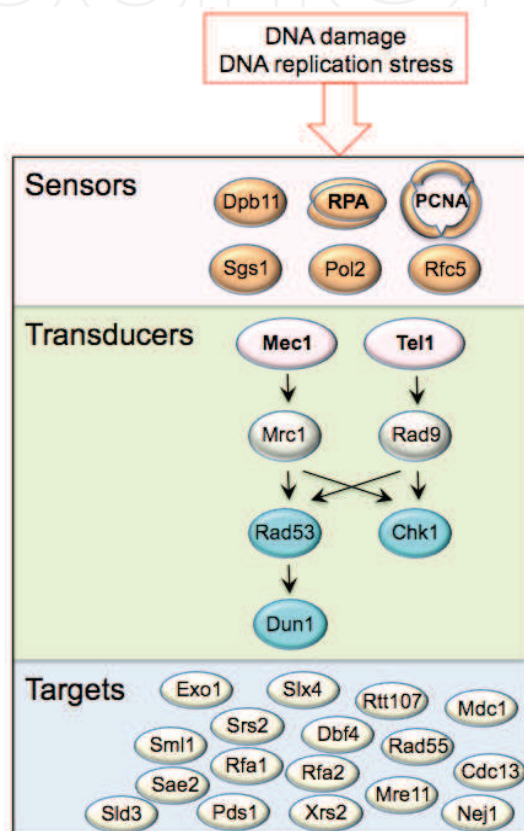


Fig. 8. Overview of the DNA damage and DNA replication checkpoints. DNA damage and DNA replication stress is sensed by a number of proteins, although the exact mechanism remains unclear and additional sensors likely exist. Two central kinases, Mec1 and Tel1, amplify and transmit the signal to downstream kinases Rad53, Dun1, and Chk1. Many targets of these kinases exist and only a selection is shown here.

#### 4.1 Cdk1 activity remains high during activation of the replication checkpoint in *S. cerevisiae*

The replication checkpoint has several important functions that help maintain cell viability during DNA replication stress. The best known function of the checkpoint is to induce cell cycle arrest (Hartwell and Weinert 1989). In contrast to higher eukaryotes, Cdk1 activity remains high during checkpoint arrest (Sorger and Murray 1992). One reason for maintaining high Cdk1 activity during S phase arrest is that Cdk1 activity, as described above, prevents re-licensing of origins that have already fired (Zegerman and Diffley 2010).

Thereby, the cell will not activate additional origins of replication in the face of replication stress, which could further threaten genome stability. Another reason for maintaining Cdk1 activity is that Cdk1 is important for repair of DNA double strand break (DSBs) that may have arisen as a result from collapsed DNA replication forks (Ira, Pellicioli et al. 2004). Here, Cdk1 phosphorylates and activates the nuclease Sae2 (Huertas, Cortes-Ledesma et al. 2008), which is required for resection of DNA double strand breaks, the first step of homologous recombination (HR). At the same time, Cdk1 actively suppresses the recruitment of proteins involved in non-homologous end-joining (NHEJ) (Zhang, Shim et al. 2009). This mechanism ensures that in G1 (when there is only one copy of the genome present in the cell and therefore no template for HR) NHEJ is the preferred mechanism of repair of DSBs, while during the cell cycle stages that Cdk1 is active (S-G2-M) HR is the preferred repair pathway.

#### **4.2 The DNA replication checkpoint enforces cell cycle arrest**

If Cdk1 remains active during activation of the DNA replication checkpoint, how then is cell cycle arrest enforced? One main mechanism of preventing cell cycle progression is the inhibition of firing of late origins of replication by Rad53 through phosphorylation of Sld3 and Dbf4 (Lopez-Mosqueda, Maas et al. 2010; Zegerman and Diffley 2010) (Fig. 9). Rad53-mediated Sld3 phosphorylation may prevent the interaction of Sld3 with Cdc45 and Dpb11, thus preventing origins from firing. However, the mechanism whereby Rad53-mediated Dbf4 phosphorylation prevents DDK from activating origins remains unknown (Lopez-Mosqueda, Maas et al. 2010; Zegerman and Diffley 2010). Rad53, in conjunction with Pds1, also prevents cell cycle progression by stabilizing Pds1 (Sanchez, Bachant et al. 1999; Agarwal, Tang et al. 2003). Pds1 is the yeast version of securin, an inhibitor of the separase Esp1. Esp1 is important for activating anaphase by cleaving cohesin, a protein complex that keeps sister chromatids together after DNA replication. Thus, stabilization of Pds1 by Rad53 and Chk1 helps prevent anaphase onset.

#### **4.3 The DNA replication checkpoint stabilizes replication forks**

A major function of the replication checkpoint is to stabilize replication forks. Stalled replication forks are at risk of collapse, which would result in partially unreplicated DNA and DNA double strand breaks, often leading to genome instability. Mec1 and Rad53 are the main players in stabilizing replication forks, while Chk1 appears to have a more redundant function (Segurado and Diffley 2008). The exact molecular mechanism remains unclear. Many substrates of Mec1 and Rad53 have been suggested (Segurado and Tercero 2009). A main target of Rad53 may be Exo1 (Segurado and Diffley 2008), although the exact molecular mechanism is not very clear. The targets of Mec1 and Chk1 in replication fork stabilization remain unknown. After the problems leading to DNA replication stress and activation of the checkpoint have been rectified, the replication forks need to restart. This process requires the activity of Rad53 and may also involve HR-dependent mechanisms (Petermann and Helleday 2010).

#### **4.4 Other processes controlled by the DNA replication checkpoint**

Although I will not go into great detail, it is worth mentioning that activation of the replication checkpoint has several other consequences. One important target of the checkpoint is Sml1, which is an inhibitor of ribonucleotide reductase, which synthesizes

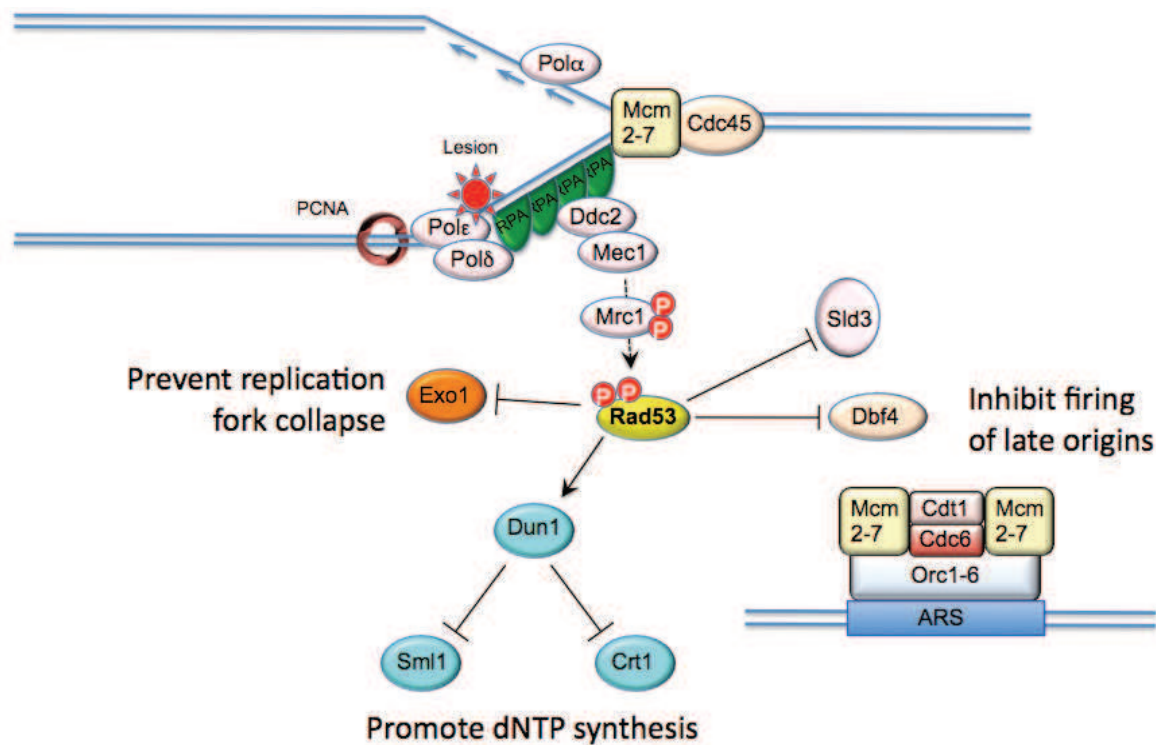


Fig. 9. Effects of the DNA replication checkpoint. When a DNA replication fork stalls due to a lesion in the DNA, the MCM2-7 helicase may continue unwinding DNA. The resulting ssDNA is bound by RPA (shown in green), which may recruit and activate Mec1. Mec1, through the replication fork-associated adaptor protein Mrc1, activates Rad53. Rad53 then inhibits firing of late origins of replication through phosphorylation and inhibition of Sld3 and Dbf4. Rad53 also protects replication fork collapse by inhibiting Exo1, and it promotes dNTP synthesis through Dun1, which phosphorylates and inhibits Sml1, which inhibits ribonucleotide reductase, and Crt1. Dun1 also inhibits Crt1, a transcriptional repressor of genes that encode ribonucleotide reductase.

dNTPs. Sml1 is phosphorylated by the checkpoint kinase Dun1, leading to destruction of Sml1 and thus increased activity of ribonucleotide reductase, which is important for sustaining cellular dNTP levels (Zhao and Rothstein 2002). Another process controlled by the checkpoint is transcription. Activation of the DNA replication checkpoint by chemicals such as MMS, which methylates DNA leading to replication fork stalling, broadly alters transcription of a large number of genes (Putnam, Jaehnig et al. 2009). Many of these genes do not confer resistance to agents that induce DNA replication stress (Putnam, Jaehnig et al. 2009). Nonetheless, there also exist specific pathways that activate transcription of genes that have important functions in maintaining cell viability during DNA replication stress. For example, Dun1 phosphorylates and inhibits the transcriptional repressor Crt1, which leads to increased transcription of the ribonucleotide reductase encoding genes *RNR1-4* (Putnam, Jaehnig et al. 2009). Finally, the replication checkpoint is important for preventing chromosome separation by targeting the mitotic spindle (Krishnan, Nirantar et al. 2004), it ensures proper cell morphogenesis during DNA replication stress (Enserink, Smolka et al. 2006; Smolka, Chen et al. 2006), maintains cellular histone levels (Gunjan and Verreault 2003), and affects migration of the nucleus (Dotiwala, Haase et al. 2007). Additional functions likely exist to maintain cell viability during DNA replication stress.

## 5. Concluding remarks

DNA replication and cell cycle control are tightly connected. The cyclin dependent kinase Cdk1 is involved both in initiation of DNA replication and in safeguarding that replication takes place once per cell cycle. Deregulated Cdk1 activity leads to inefficient DNA replication and genome instability, and checkpoints have evolved that monitor DNA replication and chromosomal integrity. While the past decade has strongly increased our understanding of the processes that control DNA replication, the details of the molecular mechanisms often remain shrouded, and it will be exciting to see this field develop.

## 6. References

- Agarwal, R., Z. Tang, et al. (2003). "Two distinct pathways for inhibiting pds1 ubiquitination in response to DNA damage." *J Biol Chem* 278(45): 45027-33.
- Alcasabas, A. A., A. J. Osborn, et al. (2001). "Mrc1 transduces signals of DNA replication stress to activate Rad53." *Nat Cell Biol* 3(11): 958-65.
- Araki, H. (2010). "Cyclin-dependent kinase-dependent initiation of chromosomal DNA replication." *Curr Opin Cell Biol* 22(6): 766-71.
- Bell, S. P. and B. Stillman (1992). "ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex." *Nature* 357(6374): 128-34.
- Booher, R. N., R. J. Deshaies, et al. (1993). "Properties of *Saccharomyces cerevisiae* wee1 and its differential regulation of p34CDC28 in response to G1 and G2 cyclins." *Embo J* 12(9): 3417-26.
- Branzei, D. and M. Foiani (2010). "Maintaining genome stability at the replication fork." *Nat Rev Mol Cell Biol* 11(3): 208-19.
- Byun, T. S., M. Pacek, et al. (2005). "Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint." *Genes Dev* 19(9): 1040-52.
- Charvin, G., C. Oikonomou, et al. (2010). "Origin of irreversibility of cell cycle start in budding yeast." *PLoS Biol* 8(1): e1000284.
- Costanzo, M., J. L. Nishikawa, et al. (2004). "CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast." *Cell* 117(7): 899-913.
- de Bruin, R. A., T. I. Kalashnikova, et al. (2006). "Constraining G1-specific transcription to late G1 phase: the MBF-associated corepressor Nrm1 acts via negative feedback." *Mol Cell* 23(4): 483-96.
- de Bruin, R. A., W. H. McDonald, et al. (2004). "Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5." *Cell* 117(7): 887-98.
- Dotiwala, F., J. Haase, et al. (2007). "The yeast DNA damage checkpoint proteins control a cytoplasmic response to DNA damage." *Proc Natl Acad Sci U S A* 104(27): 11358-63.
- Drury, L. S., G. Perkins, et al. (1997). "The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast." *EMBO J* 16(19): 5966-76.
- Enserink, J. M., H. Hombauer, et al. (2009). "Cdc28/Cdk1 positively and negatively affects genome stability in *S. cerevisiae*." *J Cell Biol* 185(3): 423-37.
- Enserink, J. M. and R. D. Kolodner (2010). "An overview of Cdk1-controlled targets and processes." *Cell Div* 5: 11.



- Enserink, J. M., M. B. Smolka, et al. (2006). "Checkpoint proteins control morphogenetic events during DNA replication stress in *Saccharomyces cerevisiae*." *J Cell Biol* 175(5): 729-41.
- Ferrell, J. E., Jr., T. Y. Tsai, et al. (2011). "Modeling the cell cycle: why do certain circuits oscillate?" *Cell* 144(6): 874-85.
- Gilbert, C. S., C. M. Green, et al. (2001). "Budding yeast Rad9 is an ATP-dependent Rad53 activating machine." *Mol Cell* 8(1): 129-36.
- Gunjan, A. and A. Verreault (2003). "A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in *S. cerevisiae*." *Cell* 115(5): 537-49.
- Hartwell, L. H. and T. A. Weinert (1989). "Checkpoints: controls that ensure the order of cell cycle events." *Science* 246(4930): 629-34.
- Huang, D., S. Kaluarachchi, et al. (2009). "Dual regulation by pairs of cyclin-dependent protein kinases and histone deacetylases controls G1 transcription in budding yeast." *PLoS Biol* 7(9): e1000188.
- Huertas, P., F. Cortes-Ledesma, et al. (2008). "CDK targets Sae2 to control DNA-end resection and homologous recombination." *Nature* 455(7213): 689-92.
- Ira, G., A. Pellicioli, et al. (2004). "DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1." *Nature* 431(7011): 1011-7.
- Jaspersen, S. L., J. F. Charles, et al. (1999). "Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14." *Curr Biol* 9(5): 227-36.
- Jeffrey, P. D., A. A. Russo, et al. (1995). "Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex." *Nature* 376(6538): 313-20.
- Koch, C., A. Schleiffer, et al. (1996). "Switching transcription on and off during the yeast cell cycle: Cln/Cdc28 kinases activate bound transcription factor SBF (Swi4/Swi6) at start, whereas Clb/Cdc28 kinases displace it from the promoter in G2." *Genes Dev* 10(2): 129-41.
- Kolodner, R. D., C. D. Putnam, et al. (2002). "Maintenance of genome stability in *Saccharomyces cerevisiae*." *Science* 297(5581): 552-7.
- Krishnan, V., S. Nirantar, et al. (2004). "DNA replication checkpoint prevents precocious chromosome segregation by regulating spindle behavior." *Mol Cell* 16(5): 687-700.
- Labib, K., J. F. Diffley, et al. (1999). "G1-phase and B-type cyclins exclude the DNA-replication factor Mcm4 from the nucleus." *Nat Cell Biol* 1(7): 415-22.
- Lee, H. O., J. M. Davidson, et al. (2009). "Endoreplication: polyploidy with purpose." *Genes Dev* 23(21): 2461-77.
- Lengronne, A. and E. Schwob (2002). "The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G(1)." *Mol Cell* 9(5): 1067-78.
- Liku, M. E., V. Q. Nguyen, et al. (2005). "CDK phosphorylation of a novel NLS-NES module distributed between two subunits of the Mcm2-7 complex prevents chromosomal rereplication." *Mol Biol Cell* 16(10): 5026-39.
- Lopez-Mosqueda, J., N. L. Maas, et al. (2010). "Damage-induced phosphorylation of Sld3 is important to block late origin firing." *Nature* 467(7314): 479-83.
- Malumbres, M. and M. Barbacid (2009). "Cell cycle, CDKs and cancer: a changing paradigm." *Nat Rev Cancer* 9(3): 153-66.

- Mimura, S., T. Seki, et al. (2004). "Phosphorylation-dependent binding of mitotic cyclins to Cdc6 contributes to DNA replication control." *Nature* 431(7012): 1118-23.
- Moll, T., G. Tebb, et al. (1991). "The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor SWI5." *Cell* 66(4): 743-58.
- Muramatsu, S., K. Hirai, et al. (2010). "CDK-dependent complex formation between replication proteins Dpb11, Sld2, Pol (epsilon), and GINS in budding yeast." *Genes Dev* 24(6): 602-12.
- Murray, A. W. and M. W. Kirschner (1989). "Dominoes and clocks: the union of two views of the cell cycle." *Science* 246(4930): 614-21.
- Nash, P., X. Tang, et al. (2001). "Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication." *Nature* 414(6863): 514-21.
- Newlon, C. S. and J. F. Theis (2002). "DNA replication joins the revolution: whole-genome views of DNA replication in budding yeast." *Bioessays* 24(4): 300-4.
- Nguyen, V. Q., C. Co, et al. (2000). "Clb/Cdc28 kinases promote nuclear export of the replication initiator proteins Mcm2-7." *Curr Biol* 10(4): 195-205.
- Nguyen, V. Q., C. Co, et al. (2001). "Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms." *Nature* 411(6841): 1068-73.
- Ostapenko, D., J. L. Burton, et al. (2008). "Pseudosubstrate inhibition of the anaphase-promoting complex by Acm1: regulation by proteolysis and Cdc28 phosphorylation." *Mol Cell Biol* 28(15): 4653-64.
- Pavletich, N. P. (1999). "Mechanisms of cyclin-dependent kinase regulation: structures of Cdk, their cyclin activators, and Cip and INK4 inhibitors." *J Mol Biol* 287(5): 821-8.
- Petermann, E. and T. Helleday (2010). "Pathways of mammalian replication fork restart." *Nat Rev Mol Cell Biol* 11(10): 683-7.
- Pines, J. (1994). "The cell cycle kinases." *Semin Cancer Biol* 5(4): 305-13.
- Putnam, C. D., E. J. Jaehnig, et al. (2009). "Perspectives on the DNA damage and replication checkpoint responses in *Saccharomyces cerevisiae*." *DNA Repair (Amst)*.
- Remus, D. and J. F. Diffley (2009). "Eukaryotic DNA replication control: lock and load, then fire." *Curr Opin Cell Biol* 21(6): 771-7.
- Russell, P. and P. Nurse (1986). "cdc25+ functions as an inducer in the mitotic control of fission yeast." *Cell* 45(1): 145-53.
- Russo, A. A., P. D. Jeffrey, et al. (1996). "Structural basis of cyclin-dependent kinase activation by phosphorylation." *Nat Struct Biol* 3(8): 696-700.
- Sanchez, Y., J. Bachant, et al. (1999). "Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms." *Science* 286(5442): 1166-71.
- Satyanarayana, A. and P. Kaldis (2009). "Mammalian cell-cycle regulation: several Cdk, numerous cyclins and diverse compensatory mechanisms." *Oncogene* 28(33): 2925-39.
- Schwob, E., T. Bohm, et al. (1994). "The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in *S. cerevisiae*." *Cell* 79(2): 233-44.
- Schwob, E. and K. Nasmyth (1993). "CLB5 and CLB6, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*." *Genes Dev* 7(7A): 1160-75.
- Segurado, M. and J. F. Diffley (2008). "Separate roles for the DNA damage checkpoint protein kinases in stabilizing DNA replication forks." *Genes Dev* 22(13): 1816-27.

- Segurado, M. and J. A. Tercero (2009). "The S-phase checkpoint: targeting the replication fork." *Biol Cell* 101(11): 617-27.
- Smolka, M. B., S. H. Chen, et al. (2006). "An FHA domain-mediated protein interaction network of Rad53 reveals its role in polarized cell growth." *J Cell Biol* 175(5): 743-53.
- Sorger, P. K. and A. W. Murray (1992). "S-phase feedback control in budding yeast independent of tyrosine phosphorylation of p34cdc28." *Nature* 355(6358): 365-8.
- Stinchcomb, D. T., K. Struhl, et al. (1979). "Isolation and characterisation of a yeast chromosomal replicator." *Nature* 282(5734): 39-43.
- Tanaka, S. and H. Araki (2010). "Regulation of the initiation step of DNA replication by cyclin-dependent kinases." *Chromosoma* 119(6): 565-74.
- Tanaka, S. and J. F. Diffley (2002). "Deregulated G1-cyclin expression induces genomic instability by preventing efficient pre-RC formation." *Genes Dev* 16(20): 2639-49.
- Theis, J. F. and C. S. Newlon (1997). "The ARS309 chromosomal replicator of *Saccharomyces cerevisiae* depends on an exceptional ARS consensus sequence." *Proc Natl Acad Sci U S A* 94(20): 10786-91.
- van den Heuvel, S. and N. J. Dyson (2008). "Conserved functions of the pRB and E2F families." *Nat Rev Mol Cell Biol* 9(9): 713-24.
- Wang, H., L. B. Carey, et al. (2009). "Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone deacetylase and other targets." *PLoS Biol* 7(9): e1000189.
- Zegerman, P. and J. F. Diffley (2010). "Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation." *Nature* 467(7314): 474-8.
- Zhang, Y., E. Y. Shim, et al. (2009). "Regulation of repair choice: Cdk1 suppresses recruitment of end joining factors at DNA breaks." *DNA Repair (Amst)* 8(10): 1235-41.
- Zhao, X. and R. Rothstein (2002). "The Dun1 checkpoint kinase phosphorylates and regulates the ribonucleotide reductase inhibitor Sml1." *Proc Natl Acad Sci U S A* 99(6): 3746-51.
- Zou, L. and S. J. Elledge (2003). "Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes." *Science* 300(5625): 1542-8.

IntechOpen



## **DNA Replication-Current Advances**

Edited by Dr Herve Seligmann

ISBN 978-953-307-593-8

Hard cover, 694 pages

**Publisher** InTech

**Published online** 01, August, 2011

**Published in print edition** August, 2011

The study of DNA advanced human knowledge in a way comparable to the major theories in physics, surpassed only by discoveries such as fire or the number zero. However, it also created conceptual shortcuts, beliefs and misunderstandings that obscure the natural phenomena, hindering its better understanding. The deep conviction that no human knowledge is perfect, but only perfectible, should function as a fair safeguard against scientific dogmatism and enable open discussion. With this aim, this book will offer to its readers 30 chapters on current trends in the field of DNA replication. As several contributions in this book show, the study of DNA will continue for a while to be a leading front of scientific activities.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jorrit M. Enserink (2011). Cell Cycle Regulation of DNA Replication in *S. cerevisiae*, DNA Replication-Current Advances, Dr Herve Seligmann (Ed.), ISBN: 978-953-307-593-8, InTech, Available from:  
<http://www.intechopen.com/books/dna-replication-current-advances/cell-cycle-regulation-of-dna-replication-in-s-cerevisiae>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](#), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen