EMBO reports

review

The chromosome cycle: coordinating replication and segregation

Second in the Cycles Review Series

J. Julian Blow[+] *& Tomoyuki U. Tanaka*
University of Dundee, Wellcome Trust Biocentre, Dundee, UK

During the cell-division cycle, chromosomal DNA must initially be precisely duplicated and then correctly segregated to daughter cells. The accuracy of these two events is maintained by two interlinked cycles: the replication licensing cycle, which ensures precise duplication of DNA, and the cohesion cycle, which ensures correct segregation. Here we provide a general overview of how these two systems are coordinated to maintain genetic stability during the cell cycle.

Keywords: cell cycle; cohesion; DNA replication; replication licensing
EMBO reports (2005) **6,** 1028–1034. doi:10.1038/sj.embor.7400557

Introduction

At the heart of the eukaryotic cell-division cycle are the precise duplication and segregation of chromosomal DNA. These two events take place at distinct points of the cell cycle: DNA is replicated during S phase, and then the replicated DNA strands are accurately partitioned to the two daughter cells during M phase. The flawless operation of this cycle is important not only for the maintenance of viability, but also for preventing genetic instability, which, in metazoans, could potentially drive tumorigenesis. In this review, we look at the cell cycle from the viewpoint of chromosomal DNA and its associated regulators, and focus on how precise chromosome duplication and segregation occur.

Similar to other cell-cycle events, chromosome duplication and segregation are chiefly driven by cyclin-dependent kinases (CDKs) and a ubiquitin ligase known as the anaphase-promoting complex or cyclosome (APC/C). Immediately after cell division, cells are in the G1 phase of the cell cycle with low CDK activity. Rising levels of S-phase CDKs induce entry into S phase, and then the M-phase CDKs drive entry into mitosis. During early M phase, sister chromatid pairs (each comprising one of the sister DNA strands plus associated proteins) are captured by microtubules that emanate from opposite ends of the cell. During late mitosis, the APC/C bound by its activator Cdc20 (Peters, 2002) triggers separation of sister chromatids, causing their segregation to opposite poles of the cell. The APC/C also degrades cyclins, thereby lowering CDK activity and promoting exit from M phase. The cell then pinches into two parts, each containing a full complement of chromosomal DNA.

From the perspective of the protein machinery that acts on it, chromosomal DNA is extremely long. This creates two organizational problems for the cell. First, all the DNA must be replicated, but no section should be replicated more than once. Second, the sister chromatids must be identified and each sent to a different daughter cell. Eukaryotes solve both problems by stably loading chromosomal DNA with proteins during G1 that allow the cell to distinguish replicated and unreplicated DNA and to determine which two DNA strands are sisters. This 'marking' allows two large-scale processes (precise chromosome replication and segregation) to emerge from small local events.

The mark involved in DNA replication comprises the Mcm2–7 complex, which is a heterohexamer of six related 'minichromosome maintenance' proteins. Mcm2–7 complexes are loaded onto sites on chromosomal DNA during late mitosis and early G1, thereby 'licensing' these sites for use as replication origins in the upcoming S phase (Blow & Laskey, 1988; Diffley, 2004; Nishitani & Lygerou, 2004; Blow & Dutta, 2005). During S phase, replication forks are initiated only at these licensed replication origins. After initiation occurs at each origin, the Mcm2–7 complex is removed and probably moves along with the replication fork. Each replication fork terminates when it encounters another heading towards it; at this stage, the Mcm2–7 complex associated with the fork is displaced from the DNA. To prevent replicated origins from becoming re-licensed and, hence, re-replicated, the ability to load new Mcm2–7 complexes onto DNA is prevented during the late G1, S, G2 and early M phases. This behaviour is outlined in Fig 1.

The mark involved in the correct segregation of sister strands is cohesin, which is a complex consisting of at least four proteins that can form a ring structure (Nasmyth & Haering, 2005). At the end of M phase or during G1, the cohesin complex is loaded

University of Dundee, Wellcome Trust Biocentre, Dow Street, Dundee DD1 5EH, UK
[+]Corresponding author. Tel: +44 1382 345797; Fax: +44 1382 348072;
E-mail: j.j.blow@dundee.ac.uk

Submitted 21 July 2005; accepted 25 August 2005

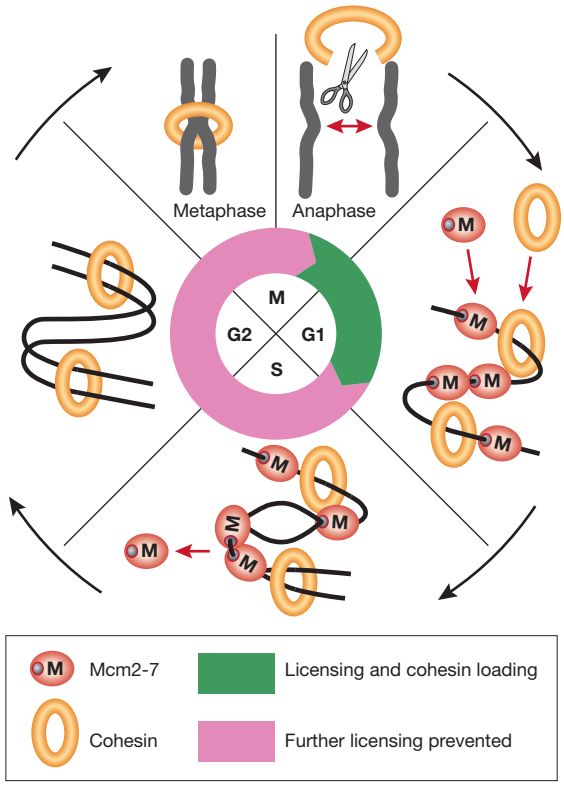


Fig 1 | Overview of the licensing and cohesion cycles. A small segment of chromosomal DNA, encompassing three replication origins, is shown during G1, S and G2. Mcm2–7 and cohesin are loaded during G1. During S phase, the Mcm2–7 complex is displaced from DNA as it replicates, and cohesion is established. During anaphase, cohesin is cleaved, thereby allowing segregation of sister chromatids.

onto DNA. During S phase, the cohesin complex establishes a physical link (cohesion) between sister chromatids. Cohesin is required for maintenance of this cohesion, and for ensuring that sister chromatids are attached to microtubules extending from opposite spindle poles (sister kinetochore bi-orientation) during the subsequent M phase (Tanaka *et al*, 2000; Sonoda *et al*, 2001). At the metaphase-to-anaphase transition, after all chromosomes bi-orientate, a protease called separase is activated; this cleaves the cohesin ring and allows the sister chromatids to be pulled apart by the mitotic spindle (Fig 1; Uhlmann, 2003). The whole cycle of licensing and cohesin loading is then able to start again.

Licensing and cohesin loading

Crystallography and electron microscopy of the archaeal Mcm2–7 homologue indicates that it forms a hexameric ring with a positively-charged central channel that is able to encircle double-stranded DNA (Fletcher *et al*, 2003; Pape *et al*, 2003). The loading of Mcm2–7 onto DNA as an origin is licensed might involve the opening of the Mcm2–7 ring to allow it to encircle the DNA. This would explain the high stability of Mcm2–7 complexes on DNA. At least three other proteins—the origin recognition complex (ORC), Cdc6 and Cdt1—are required for Mcm2–7 loading and the functional licensing of DNA. Together, ORC, Cdc6, Cdt1 and Mcm2–7 are components of the pre-replicative complex (pre-RC) of proteins that are found at replication origins during G1.

The licensing reaction has been reconstituted on the chromatin of *Xenopus* spermatozoa using purified ORC, Cdc6, Cdt1 and Mcm2–7, in addition to a chromatin-remodelling protein that is required for the initial binding of ORC to DNA (Gillespie *et al*, 2001). ORC binds to the DNA, and then recruits Cdc6 and Cdt1. ORC, Cdc6 and Cdt1 are required for the initial loading of Mcm2–7 onto DNA, but are not essential for the continued binding of Mcm2–7. This is consistent with ORC, Cdc6 and Cdt1 acting as a clamp loader that opens up the Mcm2–7 hexamer and closes it again around the DNA (Fig 2). ORC, Cdc6 and Cdt1 are able to load several Mcm2–7 hexamers onto DNA, and each replication origin (ORC-binding site) typically has 10–40 Mcm2–7 hexamers associated with it by the end of G1 (Blow & Dutta, 2005).

The cohesin complex consists of four proteins: Smc1, Smc3, Scc1 (also called Mcd1 and Rad21) and Scc3 (also called Psc3 and SA1/2; Nasmyth & Haering, 2005). Each Smc molecule forms an intramolecular antiparallel coiled-coil, which brings their globular amino (N)- and carboxy (C)-terminal domains together (Anderson *et al*, 2002; Haering *et al*, 2002). Smc1 and Smc3 dimerize through their central domains to form a V-shaped heterodimer, which is closed by Scc1 and Scc3 to form a ring structure. The cohesin complex is loaded onto chromosomes at the end of M phase in fission yeast and metazoan cells, and in late G1 in budding yeast. This loading process requires a complex consisting of Scc2 (also called Mis4 and Nipped-B) and Scc4 (Ciosk *et al*, 2000; Tomonaga *et al*, 2000). Cohesin loading also requires the ATPase activity of Smc1 and Smc3 (Arumugam *et al*, 2003; Weitzer *et al*, 2003). Similar to licensing, this loading reaction might involve opening up the cohesin ring and closing it again around the DNA. In *Xenopus* egg extracts, the recruitment of Scc2 to chromosomes depends on the presence of Mcm2–7 on the chromatin, which therefore tightly links these two processes (Gillespie & Hirano, 2004; Takahashi *et al*, 2004). However, in budding yeast, cohesin loading takes place independently of pre-RC proteins and, in contrast to Mcm2–7 loading, can even occur after DNA replication (although, in this case, without contributing to sister chromatid cohesion; Uhlmann & Nasmyth 1998; Haering *et al*, 2004). Cohesin association with chromosomes is found at preferred sites, which include the regions around centromeres and sites between convergent transcription units (Blat & Kleckner, 1999; Megee *et al*, 1999; Tanaka *et al*, 1999; Tomonaga *et al*, 2000; Bernard *et al*, 2001; Glynn *et al*, 2004; Lengronne *et al*, 2004). One possibility is that once loaded onto DNA, cohesin is redistributed to different places, perhaps by the act of transcription itself.

Downregulation of licensing in late G1

To prevent replicated DNA from becoming re-licensed and, hence, re-replicated during a single S phase, it is crucial that the ability to load new Mcm2–7 hexamers onto DNA ceases before entry into S phase. This can be achieved by downregulating the activity of the ORC–Cdc6–Cdt1 loading machinery without affecting Mcm2–7 that is already bound to DNA. Different organisms regulate ORC, Cdc6 and Cdt1 activity in a range of ways (Diffley, 2004; Nishitani & Lygerou, 2004; Blow & Dutta, 2005).

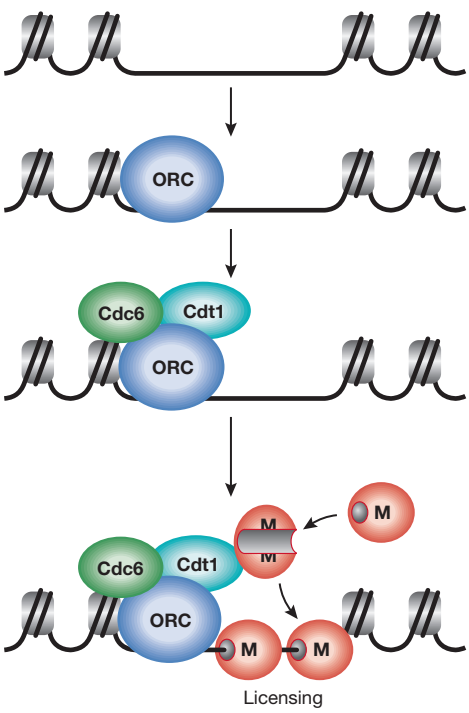


Fig 2 | Components of the pre-replicative complex. The licensing of a single replication origin is shown. ORC is first recruited to chromatin, and then recruits Cdc6 and Cdt1. The ORC–Cdc6–Cdt1 complex licenses the origin by loading numerous Mcm2–7 complexes onto it.

These can be grouped into four categories (proteolysis, inhibitory phosphorylation, nuclear export and inhibition by geminin), which we briefly examine in turn below.

Proteolysis. In yeast, rising CDK levels at the end of G1 cause phosphorylation of Cdc6 (fission yeast Cdc18), which targets it for ubiquitylation and subsequent proteolysis (Drury *et al*, 1997; Jallepalli *et al*, 1997). In metazoans, Cdc6 levels remain relatively constant throughout the cell cycle. Instead, metazoan Cdt1 is ubiquitylated and degraded at the onset of S phase, partly owing to CDK-dependent phosphorylation, and this is important in preventing re-licensing of replicated DNA (Nishitani *et al*, 2001; Li *et al*, 2003; Zhong *et al*, 2003; Arias & Walter, 2005; Li & Blow, 2005). In metazoans, ORC and Cdc6 are also regulated by ubiquitylation and proteolysis, although the physiological consequences of this regulation remain uncertain.

Inhibitory phosphorylation. In yeast, the Orc2 subunit is phosphorylated by CDKs during late G1, S, G2 and mitosis, and this has a role in preventing re-licensing of replicated DNA (Nguyen *et al*, 2001; Vas *et al*, 2001). CDK-dependent phosphorylation of the mammalian ORC and Cdc6 probably inhibits their binding to DNA during mitosis (Li *et al*, 2004).

Nuclear export. In budding yeast, but not fission yeast or metazoans, CDK activity leads to the nuclear export of both Mcm2–7 (Tanaka *et al*, 1997; Labib *et al*, 1999) and Cdt1. This prevents these proteins from gaining access to their DNA substrate and so prevents re-licensing of replicated DNA. Export of Mcm2–7 and Cdt1 is interdependent, and might be driven by phosphorylation of Mcm2–7.

Inhibition by geminin. Geminin is a small protein that binds and inhibits Cdt1 (McGarry & Kirschner, 1998; Wohlschlegel *et al*, 2000; Tada *et al*, 2001). So far, it has been described only in metazoans. Geminin is ubiquitylated by the APC/C during late mitosis and early G1. In somatic cells, this action targets it for proteolysis; however, in early embryos, ubiquitylation of geminin leads to its inactivation rather than its degradation (Hodgson *et al*, 2002). Levels of active geminin are high during S, G2 and mitosis, and this is important in preventing re-licensing of replicated DNA (Quinn *et al*, 2001; Mihaylov *et al*, 2002; Melixetian *et al*, 2004; Zhu *et al*, 2004; Li & Blow, 2005).

Directly or indirectly, these inhibitory mechanisms ultimately depend on the increase in CDK activity that occurs at the end of G1 and persists until late mitosis (Diffley, 2004; Nishitani & Lygerou, 2004; Blow & Dutta, 2005). The cell cycle can, therefore, be thought of as consisting of two phases. The first phase comprises late mitosis and early G1, when CDK levels are low and licensing and cohesin loading occur. The second phase comprises late G1, S, G2 and early mitosis, when high CDK levels drive DNA replication and chromosome segregation (Fig 1).

In budding yeast, these different inhibitory pathways are redundant; to achieve re-replication of DNA, it is necessary to stabilize Cdc6, remove inhibitory phosphorylation sites on ORC, and prevent nuclear export of Mcm2–7 and Cdt1 (Nguyen *et al*, 2001). In metazoans, downregulation of Cdt1 alone seems to be the most important mechanism that prevents re-licensing of replicated DNA, as overexpression of Cdt1 or elimination of geminin can cause extensive re-replication (Quinn *et al*, 2001; Mihaylov *et al*, 2002; Vaziri *et al*, 2003; Zhong *et al*, 2003; Melixetian *et al*, 2004; Zhu *et al*, 2004; Li & Blow, 2005). It is curious that metazoans, in which the consequences of genetic instability are greater (as it might cause cancer), do not rely on the numerous mechanisms that are used by budding yeast. One possible explanation is that this might make it harder for genetically unstable clones to arise. If geminin were only one of several redundant mechanisms, its deletion might lead to clones that occasionally re-replicate segments of DNA; however, being essential for the prevention of re-replication, geminin deletion is instead lethal.

S phase

Mcm2–7 proteins are essential for replication of DNA during S phase. They have sequence similarity with known DNA helicases (DNA-unwinding enzymes) and show DNA helicase activity *in vitro* (Blow & Dutta, 2005). Chromatin-immunoprecipitation experiments in yeast have shown that during S phase, Mcm2–7 proteins are displaced from replication origins and move ahead of the replication fork (Aparicio *et al*, 1997; Tanaka *et al*, 1997). If Mcm2–7 function is inhibited during S phase, the rate of DNA synthesis rapidly falls, which indicates that Mcm2–7 proteins are required for the progression of replication forks (Labib *et al*, 2000; Pacek & Walter, 2004; Shechter *et al*, 2004). If Mcm2–7 proteins form a replicative helicase that unwinds the template DNA ahead of the replication fork, this explains why they are displaced from replication origins when fork initiation occurs, thereby rendering the origin unlicensed once it has been initiated.

One question left unanswered by this model is why so many Mcm2–7 hexamers are loaded onto the DNA (10–40 per origin). One possible explanation is that Mcm2–7 can act at a distance from the replication fork, pumping the DNA into the fork and

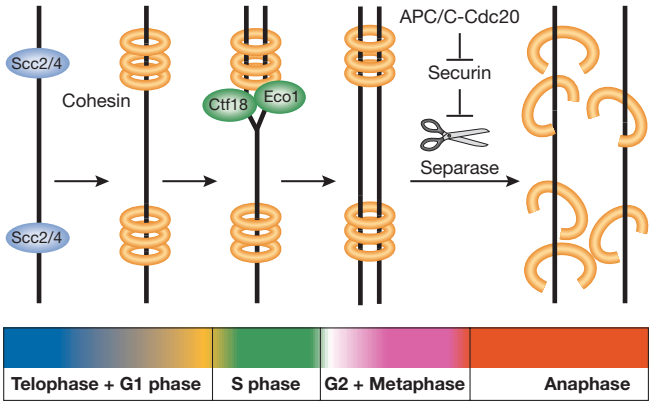


Fig 3 | The cohesin cycle. During telophase or G1, cohesin loading depends on Scc2 and Scc4. During S phase, establishment of cohesion requires Ctf18, Eco1 and other proteins, as well as cohesin. At the onset of anaphase, APC/C–Cdc20 promotes degradation of securin, thereby activating separase, which cleaves cohesin.

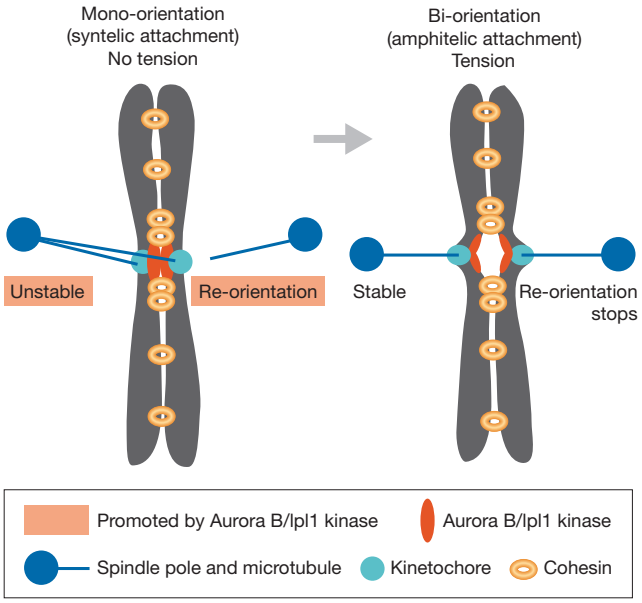


Fig 4 | Ensuring sister kinetochore bi-orientation. In the absence of the tension across sister kinetochores, Aurora B kinase (called Ipl1 in budding yeast) destabilizes the interaction between microtubules and kinetochores (left). Once bi-orientation is established, tension across the kinetochores (dependent on the presence of cohesion) stabilizes the microtubule–kinetochore interaction (right).

thereby untwisting the DNA (Laskey & Madine, 2003). Another possibility is that the excess Mcm2–7 proteins have some other function, perhaps being involved in cell-cycle-checkpoint signalling or being used only under certain conditions. Consistent with this idea is the observation that the number of Mcm2–7 complexes can be lowered to approximately two per replication origin without significantly affecting the overall replication rate (Mahbubani *et al*, 1997).

As DNA is replicated, Mcm2–7 proteins are displaced from it. The release of Mcm2–7 proteins from DNA presumably occurs when replication forks terminate as they encounter another such fork coming from the opposite direction. Little is known about this process, which involves the disassembly of the replication-fork machinery. It is possible that termination is driven by the release of Mcm2–7 proteins from DNA.

Another circumstance under which Mcm2–7 proteins should be released from DNA is the passive replication of unfired replication origins (Blow & Laskey, 1988). Not all licensed replication origins initiate during each cell cycle, rather they can be passively replicated by forks emanating from adjacent replication origins. Mcm2–7 proteins must be displaced from these uninitiated origins as they are replicated to prevent the replicated origin from being initiated later in the same S phase and, hence, causing re-replication of the surrounding DNA.

Cohesion between sister chromatids is established during S phase (Uhlmann & Nasmyth, 1998), probably as soon as the DNA is replicated. Although cohesin is required for the establishment of cohesion, the loading of cohesin onto chromosomes is not sufficient for this purpose (Nasmyth & Haering, 2005; Skibbens, 2005). The establishment of cohesion requires several other factors, including Eco1 (also called Ctf7 and Eso1), Ctf4 and a variant of replication factor C containing Ctf18 (Skibbens *et al*, 1999; Toth *et al*, 1999; Hanna *et al*, 2001; Mayer *et al*, 2001). Loss of these factors causes defects in sister chromatid cohesion but not in the association of cohesin with chromosomes. Little is known about how cohesin structurally contributes to sister chromatid cohesion, but it is interesting that several of the proteins that are required for cohesion establishment might function at the replication fork. One possibility is that the cohesin ring establishes cohesion by embracing both sister chromatids (as implied in Fig 3), although alternative models have also been proposed (Nasmyth & Haering, 2005; Skibbens, 2005).

Mitosis

In many eukaryotic cells, including those of vertebrates (but not budding yeast), most cohesin dissociates from chromosomes during prophase. This process is probably required for the resolution of sister chromatids, and is facilitated by the Polo-like and Aurora B kinases (Losada *et al*, 2002; Sumara *et al*, 2002). Polo-like kinase, which is activated as CDK levels rise at the onset of M phase, promotes cohesin dissociation by phosphorylating Scc3 (Hauf *et al*, 2005). After the bulk of cohesin is dissociated from the chromosome arms, cohesin is retained between sister centromeres until the onset of anaphase (Waizenegger *et al*, 2000). This centromeric retention of cohesin is dependent on shugosin (Mei-S332 in *Drosophila*; LeBlanc *et al*, 1999; Salic *et al*, 2004; Kitajima *et al*, 2005; McGuinness *et al*, 2005). In addition, the cohesin-loading factor Scc2 is removed from DNA as a consequence of high CDK activity in *Xenopus* extract (Gillespie & Hirano, 2004).

For proper chromosome segregation, kinetochores assembled onto the centromeric DNA of sister chromatids must attach to microtubules extending from opposite spindle poles before the onset of anaphase (Tanaka, 2005). This state is called sister kinetochore bi-orientation or amphitelic attachment. If sister kinetochores incorrectly orientate on the mitotic spindle—for instance, if both attach to microtubules from the same spindle

pole—then the Aurora B kinase facilitates re-orientation of kinetochore–microtubule connections by phosphorylating kinetochore components (Cheeseman *et al*, 2002; Tanaka *et al*, 2002; Hauf *et al*, 2003; Lampson *et al*, 2004; Fig 4). This process continues until proper bi-orientation occurs, at which stage tension is generated across the two sister kinetochores as they are pulled by microtubules to opposite spindle poles (Nicklas, 1997; Dewar *et al*, 2004). The presence of tension across the two kinetochores, which depends on cohesin, might prevent Aurora B from further re-orientating the kinetochore–microtubule interaction. In the absence of cohesin, half the chromosomes fail to bi-orientate on the spindle, although kinetochores are still able to attach to microtubules on such chromosomes (Tanaka *et al*, 2000; Sonoda *et al*, 2001). In this way, sister chromatid cohesion ensures the precise segregation of sister chromatids to the opposite sides of the cell.

At the metaphase-to-anaphase transition, the separation of sister chromatids is triggered by the removal of the residual (in vertebrate cells) or bulk (in budding yeast) cohesin from chromosomes (Uhlmann, 2003; Nasmyth & Haering, 2005). As the separation of sister chromatids is irreversible, a number of fail-safe mechanisms regulate this step. A chief regulator is a protease termed separase (Fig 3), which cleaves the cohesin Scc1 and thereby opens up the cohesin-ring structure (Uhlmann *et al*, 2000). Separase activity is inhibited by a protein called securin, and the activation of separase at the metaphase-to-anaphase transition depends on the degradation of securin by the proteasome (Cohen-Fix *et al*, 1996; Funabiki *et al*, 1996; Ciosk *et al*, 1998). This is facilitated by polyubiquitylation of securin by the APC/C bound by its activator Cdc20. Moreover, at least in vertebrate cells, mitotic CDKs are able to phosphorylate separase, which leads to the inhibition of its activity during early mitosis (Stemmann *et al*, 2001). Therefore, separase activation also requires the degradation of M-phase cyclins, which is also facilitated by the APC/C. In addition, Polo-like kinases phosphorylate Scc1 during M phase, which renders it more susceptible to cleavage by separase (Alexandru *et al*, 2001; Hauf *et al*, 2005). In case kinetochores fail to attach to microtubules or to bi-orientate on the spindle, the Mad2 protein binds and inhibits Cdc20, and thereby delays the anaphase onset until all kinetochores properly bi-orientate on the spindle (the 'spindle checkpoint'; Lew & Burke, 2003).

On exit from mitosis, the licensing system needs to be reactivated. This is achieved by the inactivation of geminin (in metazoans) and CDKs, which occurs as a consequence of APC/C activation. Reduction of CDK activity increases the affinity of ORC and Cdc6 for chromatin (Tada *et al*, 2001; Li *et al*, 2004), and, in budding yeast, allows Mcm2–7 and Cdt1 to re-enter the nucleus (Labib *et al*, 1999). In metazoans, polyubiquitylation of geminin by the APC/C leads to its proteolysis (McGarry & Kirschner, 1998) or inactivation (Hodgson *et al*, 2002). As both geminin and the cyclins are substrates of the APC/C, which is also responsible for cohesin cleavage, the licensing and cohesin cycles are tightly linked. The behaviour of the licensing and cohesin systems therefore ensures the coordination of chromosome replication and segregation during the cell cycle.

ACKNOWLEDGEMENTS

Thanks to N. Perkins, P. Gillespie, A. Li and A. Woodward for comments on the manuscript. J.J.B. is supported by Cancer Research UK (CR-UK) grant C303/A3135. T.U.T. is supported by The Wellcome Trust and CR-UK.

REFERENCES

Alexandru G, Uhlmann F, Mechtler K, Poupart MA, Nasmyth K (2001) Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast. *Cell* **105:** 459–472

Anderson DE, Losada A, Erickson HP, Hirano T (2002) Condensin and cohesin display different arm conformations with characteristic hinge angles. *J Cell Biol* **156:** 419–424

Aparicio OM, Weinstein DM, Bell SP (1997) Components and dynamics of DNA replication complexes in S. cerevisiae: redistribution of MCM proteins and Cdc45p during S phase. *Cell* **91:** 59–69

Arias EE, Walter JC (2005) Replication-dependent destruction of Cdt1 limits DNA replication to a single round per cell cycle in *Xenopus* egg extracts. *Genes Dev* **19:** 114–126

Arumugam P, Gruber S, Tanaka K, Haering CH, Mechtler K, Nasmyth K (2003) ATP hydrolysis is required for cohesin's association with chromosomes. *Curr Biol* **13:** 1941–1953

Bernard P, Maure JF, Partridge JF, Genier S, Javerzat JP, Allshire RC (2001) Requirement of heterochromatin for cohesion at centromeres. *Science* **294:** 2539–2542

Blat Y, Kleckner N (1999) Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell* **98:** 249–259

Blow JJ, Dutta A (2005) Preventing re-replication of chromosomal DNA. *Nature Rev Mol Cell Biol* **6:** 476–486

Blow JJ, Laskey RA (1988) A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature* **332:** 546–548

Cheeseman IM, Anderson S, Jwa M, Green EM, Kang J, Yates JR, Chan CS, Drubin DG, Barnes G (2002) Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell* **111:** 163–172

Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, Nasmyth K (2000) Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. *Mol Cell* **5:** 243–254

Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K (1998) An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell* **93:** 1067–1076

Cohen-Fix O, Peters JM, Kirschner MW, Koshland D (1996) Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev* **10:** 3081–3093

Dewar H, Tanaka K, Nasmyth K, Tanaka TU (2004) Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. *Nature* **428:** 93–97

Diffley JF (2004) Regulation of early events in chromosome replication. *Curr Biol* **14:** R778–R786

Drury LS, Perkins G, Diffley JF (1997) The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast. *EMBO J* **16:** 5966–5976

Fletcher RJ, Bishop BE, Leon RP, Sclafani RA, Ogata CM, Chen XS (2003) The structure and function of MCM from archaeal M. thermoautotrophicum. *Nature Struct Biol* **10:** 160–167

Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yanagida M (1996) Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature* **381:** 438–441

Gillespie PJ, Hirano T (2004) Scc2 couples replication licensing to sister chromatid cohesion in *Xenopus* egg extracts. *Curr Biol* **14:** 1598–1603

Gillespie PJ, Li A, Blow JJ (2001) Reconstitution of licensed replication origins on *Xenopus* sperm nuclei using purified proteins. *BMC Biochem* **2:** 15

Glynn EF, Megee PC, Yu HG, Mistrot C, Unal E, Koshland DE, DeRisi JL, Gerton JL (2004) Genome-wide mapping of the cohesin complex in the yeast Saccharomyces cerevisiae. *PLoS Biol* **2:** E259

Haering CH, Lowe J, Hochwagen A, Nasmyth K (2002) Molecular architecture of SMC proteins and the yeast cohesin complex. *Mol Cell* **9:** 773–788

Haering CH, Schoffnegger D, Nishino T, Helmhart W, Nasmyth K, Lowe J (2004) Structure and stability of cohesin's Smc1–Kleisin interaction. *Mol Cell* **15:** 951–964

Hanna JS, Kroll ES, Lundblad V, Spencer FA (2001) Saccharomyces cerevisiae CTF18 and CTF4 are required for sister chromatid cohesion. *Mol Cell Biol* **21:** 3144–3158

Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, Heckel A, Van Meel J, Rieder CL, Peters JM (2003) The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J Cell Biol* **161:** 281–294

Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters JM (2005) Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. *PLoS Biol* **3:** e69

Hodgson B, Li A, Tada S, Blow JJ (2002) Geminin becomes activated as an inhibitor of Cdt1/RLF-B following nuclear import. *Curr Biol* **12:** 678–683

Jallepalli PV, Brown GW, Muzi Falconi M, Tien D, Kelly TJ (1997) Regulation of the replication initiator protein p65(cdc18) by CDK phosphorylation. *Genes Dev* **11:** 2767–2779

Kitajima TS, Hauf S, Ohsugi M, Yamamoto T, Watanabe Y (2005) Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. *Curr Biol* **15:** 353–359

Labib K, Diffley JFX, Kearsey SE (1999) G1-phase and B-type cyclins exclude the DNA-replication factor MCM4 from the nucleus. *Nature Cell Biol* **1:** 415–422

Labib K, Tercero JA, Diffley JFX (2000) Uninterrupted MCM2-7 function required for DNA replication fork progression. *Science* **288:** 1643–1647

Lampson MA, Renduchitala K, Khodjakov A, Kapoor TM (2004) Correcting improper chromosome-spindle attachments during cell division. *Nature Cell Biol* **6:** 232–237

Laskey RA, Madine MA (2003) A rotary pumping model for helicase function of MCM proteins at a distance from replication forks. *EMBO Rep* **4:** 26–30

LeBlanc HN, Tang TT, Wu JS, Orr-Weaver TL (1999) The mitotic centromeric protein MEI-S332 and its role in sister-chromatid cohesion. *Chromosoma* **108:** 401–411

Lengronne A, Katou Y, Mori S, Yokobayashi S, Kelly GP, Itoh T, Watanabe Y, Shirahige K, Uhlmann F (2004) Cohesin relocation from sites of chromosomal loading to places of convergent transcription. *Nature* **430:** 573–578

Lew DJ, Burke DJ (2003) The spindle assembly and spindle position checkpoints. *Annu Rev Genet* **37:** 251–282

Li A, Blow JJ (2005) Cdt1 downregulation by proteolysis and geminin inhibition prevents DNA re-replication in *Xenopus*. *EMBO J* **24:** 395–404

Li CJ, Vassilev A, DePamphilis ML (2004) Role for Cdk1 (Cdc2)/cyclin A in preventing the mammalian origin recognition complex's largest subunit (Orc1) from binding to chromatin during mitosis. *Mol Cell Biol* **24:** 5875–5886

Li X, Zhao Q, Liao R, Sun P, Wu X (2003) The SCF(Skp2) ubiquitin ligase complex interacts with the human replication licensing factor Cdt1 and regulates Cdt1 degradation. *J Biol Chem* **278:** 30854–30858

Losada A, Hirano M, Hirano T (2002) Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. *Genes Dev* **16:** 3004–3016

Mahbubani HM, Chong JP, Chevalier S, Thömmes P, Blow JJ (1997) Cell cycle regulation of the replication licensing system: involvement of a Cdk-dependent inhibitor. *J Cell Biol* **136:** 125–135

Mayer ML, Gygi SP, Aebersold R, Hieter P (2001) Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in S. cerevisiae. *Mol Cell* **7:** 959–970

McGarry TJ, Kirschner MW (1998) Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* **93:** 1043–1053

McGuinness BE, Hirota T, Kudo NR, Peters JM, Nasmyth K (2005) Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. *PLoS Biol* **3:** e86

Megee PC, Mistrot C, Guacci V, Koshland D (1999) The centromeric sister chromatid cohesion site directs Mcd1p binding to adjacent sequences. *Mol Cell* **4:** 445–450

Melixetian M, Ballabeni A, Masiero L, Gasparini P, Zamponi R, Bartek J, Lukas J, Helin K (2004) Loss of Geminin induces rereplication in the presence of functional p53. *J Cell Biol* **165:** 473–482

Mihaylov IS, Kondo T, Jones L, Ryzhikov S, Tanaka J, Zheng J, Higa LA, Minamino N, Cooley L, Zhang H (2002) Control of DNA replication and chromosome ploidy by geminin and cyclin A. *Mol Cell Biol* **22:** 1868–1880

Nasmyth K, Haering CH (2005) The structure and function of smc and kleisin complexes. *Annu Rev Biochem* **74:** 595–648

Nguyen VQ, Co C, Li JJ (2001) Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature* **411:** 1068–1073

Nicklas RB (1997) How cells get the right chromosomes. *Science* **275:** 632–637

Nishitani H, Lygerou Z (2004) DNA replication licensing. *Frontiers Biosci* **9:** 2115–2132

Nishitani H, Taraviras S, Lygerou Z, Nishimoto T (2001) The human licensing factor for DNA replication Cdt1 accumulates in G1 and is destabilized after initiation of S-phase. *J Biol Chem* **276:** 44905–44911

Pacek M, Walter JC (2004) A requirement for MCM7 and Cdc45 in chromosome unwinding during eukaryotic DNA replication. *EMBO J* **23:** 3667–3676

Pape T, Meka H, Chen S, Vicentini G, van Heel M, Onesti S (2003) Hexameric ring structure of the full-length archaeal MCM protein complex. *EMBO Rep* **4:** 1079–1083

Peters JM (2002) The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol Cell* **9:** 931–943

Quinn LM, Herr A, McGarry TJ, Richardson H (2001) The *Drosophila* Geminin homolog: roles for Geminin in limiting DNA replication, in anaphase and in neurogenesis. *Genes Dev* **15:** 2741–2754

Salic A, Waters JC, Mitchison TJ (2004) Vertebrate shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis. *Cell* **118:** 567–578

Shechter D, Ying CY, Gautier J (2004) DNA unwinding is an Mcm complex-dependent and ATP hydrolysis-dependent process. *J Biol Chem* **279:** 45586–45593

Skibbens RV (2005) Unzipped and loaded: the role of DNA helicases and RFC clamp-loading complexes in sister chromatid cohesion. *J Cell Biol* **169:** 841–846

Skibbens RV, Corson LB, Koshland D, Hieter P (1999) Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes Dev* **13:** 307–319

Sonoda E, Matsusaka T, Morrison C, Vagnarelli P, Hoshi O, Ushiki T, Nojima K, Fukagawa T, Waizenegger IC, Peters JM et al (2001) Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. *Dev Cell* **1:** 759–770

Stemmann O, Zou H, Gerber SA, Gygi SP, Kirschner MW (2001) Dual inhibition of sister chromatid separation at metaphase. *Cell* **107:** 715–726

Sumara I, Vorlaufer E, Stukenberg PT, Kelm O, Redemann N, Nigg EA, Peters JM (2002) The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. *Mol Cell* **9:** 515–525

Tada S, Li A, Maiorano D, Mechali M, Blow JJ (2001) Repression of origin assembly in metaphase depends on inhibition of RLF-B/Cdt1 by geminin. *Nature Cell Biol* **3:** 107–113

Takahashi TS, Yiu P, Chou MF, Gygi S, Walter JC (2004) Recruitment of *Xenopus* Scc2 and cohesin to chromatin requires the pre-replication complex. *Nature Cell Biol* **6:** 991–996

Tanaka T, Cosma MP, Wirth K, Nasmyth K (1999) Identification of cohesin association sites at centromeres and along chromosome arms. *Cell* **98:** 847–858

Tanaka T, Fuchs J, Loidl J, Nasmyth K (2000) Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. *Nature Cell Biol* **2:** 492–499

Tanaka T, Knapp D, Nasmyth K (1997) Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. *Cell* **90:** 649–660

Tanaka TU (2005) Chromosome bi-orientation on the mitotic spindle. *Phil Trans R Soc Lond B Biol Sci* **360:** 581–589

Tanaka TU, Rachidi N, Janke C, Pereira G, Galova M, Schiebel E, Stark MJ, Nasmyth K (2002) Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell* **108:** 317–329

Tomonaga T, Nagao K, Kawasaki Y, Furuya K, Murakami A, Morishita J, Yuasa T, Sutani T, Kearsey SE, Uhlmann F, et al (2000) Characterization of fission yeast cohesin: essential anaphase proteolysis of Rad21 phosphorylated in the S phase. *Genes Dev* **14:** 2757–2770

Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K (1999) Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes Dev* **13:** 320–333

Uhlmann F (2003) Chromosome cohesion and separation: from men and molecules. *Curr Biol* **13:** R104–R114

Uhlmann F, Nasmyth K (1998) Cohesion between sister chromatids must be established during DNA replication. *Curr Biol* **8:** 1095–1101

Uhlmann F, Wernic D, Poupart MA, Koonin EV, Nasmyth K (2000) Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell* **103:** 375–386

Vas A, Mok W, Leatherwood J (2001) Control of DNA rereplication via Cdc2 phosphorylation sites in the origin recognition complex. *Mol Cell Biol* **21:** 5767–5777

Vaziri C, Saxena S, Jeon Y, Lee C, Murata K, Machida Y, Wagle N, Hwang DS, Dutta A (2003) A p53-dependent checkpoint pathway prevents rereplication. *Mol Cell* **11:** 997–1008

Waizenegger IC, Hauf S, Meinke A, Peters JM (2000) Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell* **103:** 399–410

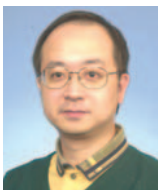
Weitzer S, Lehane C, Uhlmann F (2003) A model for ATP hydrolysis-dependent binding of cohesin to DNA. *Curr Biol* **13:** 1930–1940

Wohlschlegel JA, Dwyer BT, Dhar SK, Cvetic C, Walter JC, Dutta A (2000) Inhibition of eukaryotic replication by geminin binding to Cdt1. *Science* **290:** 2309–2312

Zhong W, Feng H, Santiago FE, Kipreos ET (2003) CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. *Nature* **423:** 885–889

Zhu W, Chen Y, Dutta A (2004) Rereplication by depletion of geminin is seen regardless of p53 status and activates a G2/M checkpoint. *Mol Cell Biol* **24:** 7140–7150

J. Julian Blow



Tomoyuki U. Tanaka

CYCLES REVIEW SERIES

See other issues of *EMBO reports* for the complete Cycles Review Series:

Organisms
The circadian cycle
by Martha Merrow, Kamiel Spoelstra & Till Roenneberg

Cells
The chromosome cycle
by J. Julian Blow & Tomoyuki U. Tanaka

Receptors
GLUT4 recycling
by Chandrasagar B. Dugani & Amira Klip

Molecules
Cycling in ATPase
by Peter Dimroth