Mechanisms of DNA replication termination

James M. Dewar[1] and Johannes C. Walter[2,3]

Abstract | Genome duplication is carried out by pairs of replication forks that assemble at origins of replication and then move in opposite directions. DNA replication ends when converging replication forks meet. During this process, which is known as replication termination, DNA synthesis is completed, the replication machinery is disassembled and daughter molecules are resolved. In this Review, we outline the steps that are likely to be common to replication termination in most organisms, namely, fork convergence, synthesis completion, replisome disassembly and decatenation. We briefly review the mechanism of termination in the bacterium *Escherichia coli* and in simian virus 40 (SV40) and also focus on recent advances in eukaryotic replication termination. In particular, we discuss the recently discovered E3 ubiquitin ligases that control replisome disassembly in yeast and higher eukaryotes, and how their activity is regulated to avoid genome instability.

Origin of DNA replication
(Origin). The location at which replicative helicases are loaded onto DNA, which are generally site-specific in bacteria and yeast, but not in metazoa.

Replication forks
Splayed DNA structures where the replisome is engaged in DNA synthesis.

[1]*Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37323, USA.*
[2]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA.*
[3]*Howard Hughes Medical Institute, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA.*
Correspondence to J.C.W. johannes_walter@hms.harvard.edu

doi:10.1038/nrm.2017.42
Published online 24 May 2017

Genomic DNA replication can be divided into three general phases: first, initiation, in which the origin of DNA replication is unwound by the replicative DNA helicase (FIG. 1a,b); second, elongation, in which replication forks copy the chromosome using semi-conservative DNA synthesis (FIG. 1c,d); and third, termination, when converging replication forks meet (FIG. 1d–g). From bacteria to eukaryotic cells, replication initiation is regulated such that genome duplication is limited to a single round per cell cycle[1,2]. Unlike initiation and elongation, which have been extensively studied[3,4], replication termination has received relatively little attention, especially in eukaryotic cells. This is a major gap in our knowledge of genome duplication, especially because termination events are as abundant as initiations, occurring approximately 50,000 times during a typical S phase of mammalian cells[5].

At least five processes are unique to the final phase of replication and thus can be considered part of replication termination. The first process concerns the resolution of topological stress. Unwinding of the parental duplex leads to overwinding of the unreplicated DNA, resulting in the formation of positive supercoils ahead of the fork (FIG. 1c). If too many supercoils accumulate, further unwinding becomes energetically unfavourable and replication ceases. There are two methods for dissipating positive supercoils. The first method involves the relaxation of supercoils by type I or type II DNA topoisomerases[6]. Alternatively, the entire fork can rotate clockwise relative to the direction of fork movement. This rotation counteracts the overwinding of unreplicated DNA and causes the two replicated sisters to cross over each other, leading to the formation of pre-catenanes[7,8] (FIG. 1e), which can be resolved by type II, but not by type I, topoisomerases. As replication proceeds, the region of parental DNA that can be supercoiled decreases in size, whereas the region of replicated DNA that can undergo pre-catenation increases. If supercoils and pre-catenanes are energetically equivalent, their relative abundance during replication should reflect the ratio of unreplicated versus replicated DNA in a topologically constrained domain[9]. In this view, as replication progresses, the resolution of topological stress would become increasingly reliant on the formation and the subsequent removal of pre-catenanes. Importantly, at some stage, the parental DNA between converging forks becomes too short to supercoil (FIG. 1d) owing to the inherent stiffness of DNA. At this stage, which occurs when 150 bp or less of parental DNA remains[10], relief of topological stress becomes dependent on the formation of pre-catenanes (FIG. 1e). This phase of replication is unique to termination and is defined as replication fork convergence. An important question is whether replication forks slow down or require accessory factors as replication becomes dependent on the formation of pre-catenanes to manage topological stress. If so, one might expect a gradual slowing of DNA replication forks as they approach one another. Moreover, if the formation or the removal of pre-catenanes were disrupted, forks would stall at a very late stage of replication owing to the accumulation of topological stress.

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

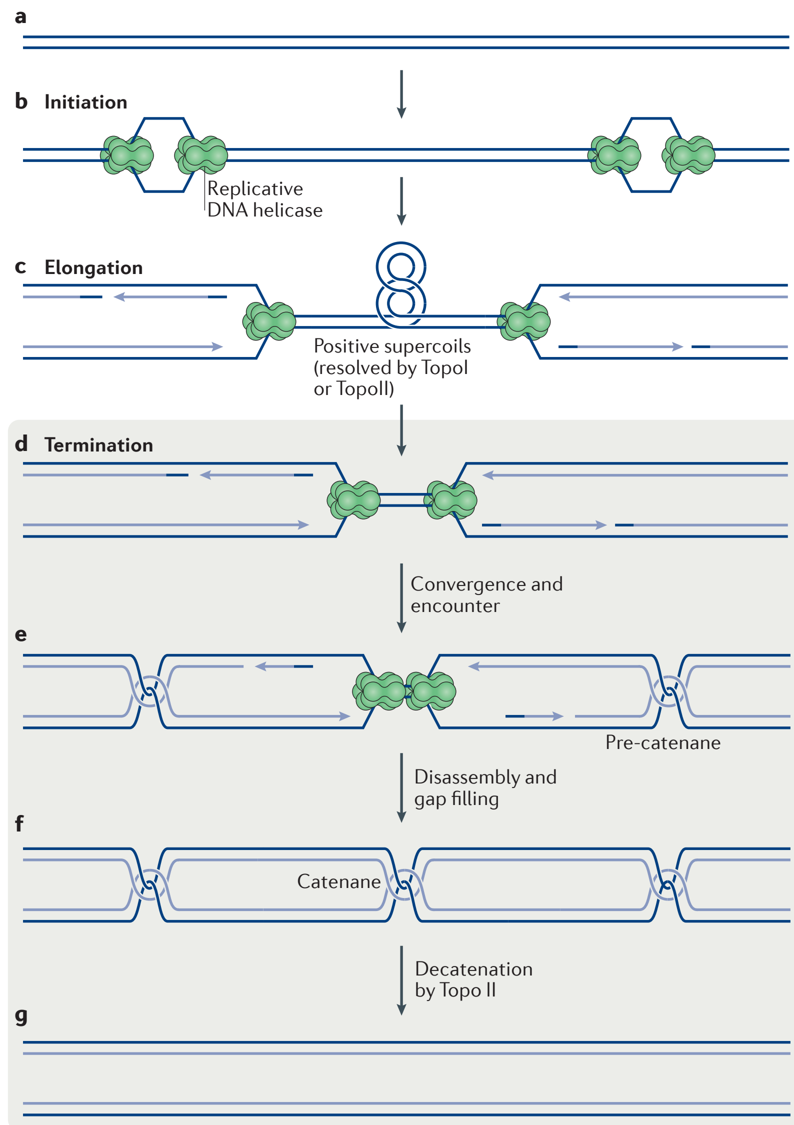


Figure 1 | **Steps in DNA replication.** Generic illustration of replication initiation (parts **a**,**b**), elongation (parts **c**,**d**) and five events that are unique to replication termination (parts **d**–**g**). The replicative DNA helicase is depicted without reference to a specific translocation mechanism; RNA primers are shown in dark blue. The order of the termination events is hypothetical. Topo, topoisomerase.

Topological stress
A structural distortion of the DNA that is caused when the two strands of the double helix are wrapped around each other too many or too few times.

Supercoils
Superhelical twists of the DNA duplex that arise in response to topological stress.

Topoisomerases
Enzymes that relieve topological stress on DNA by cutting and resealing one (type I) or both (type II) DNA strands.

The second process that is unique to replication termination is the meeting of converging replication forks, which is known as 'encounter' (FIG. 1e). It is presently unclear whether this process involves a steric clash between replisomes or whether such a clash impedes further steps in termination. Third, replisomes dissociate from the DNA in a process known as disassembly (FIG. 1e,f). It is generally assumed that the replisome dissociates during termination to prevent re-replication and to avoid interference with other chromatin-based processes, such as transcription or the next round of replication. Active disassembly pathways are likely to be required because key replisome components, such as replicative helicases and processivity factors, are clamped tightly around DNA. Crucially, to prevent fork stalling, any disassembly mechanism must not act on replisomes that are still engaged in replication. Thus, key questions include whether the replisome is actively disassembled, when this disassembly occurs and the effects of defective disassembly. Fourth, DNA synthesis is completed through gap filling (FIG. 1e,f). At replisome encounter, a single-stranded gap exists between the 3′ end of the leading strand and the downstream Okazaki fragment of the opposing fork. This gap is filled, and the last Okazaki fragment is processed. Currently, it is unclear whether gap filling requires replisome disassembly or whether the maturation of the last Okazaki fragment occurs via the same mechanism as that which occurs during replication elongation. Finally, copying the last turn of the parental duplex creates a new catenane and also converts any pre-catenanes into catenanes (FIG. 1f). All of these catenanes must be decatenated (resolved) before chromosome segregation (FIG. 1f,g). Another important issue is whether most termination events are sequence specific or stochastic, and whether these two modes are mechanistically distinct. Furthermore, the exact order of these processes during termination remains unclear.

In this Review, we first summarize the current models of termination in the bacterium *Escherichia coli* and in simian virus 40 (SV40). We then discuss recent advances in our understanding of replication termination in eukaryotes, including the first evidence of an active replisome-unloading mechanism.

Replication termination in *Escherichia coli*

The circular *E. coli* chromosome, which comprises 4.6 million base pairs of DNA, is replicated from a single origin of replication, oriC[2] (FIG. 2A). Two forks are established, each containing a hexameric replicative helicase, DNA synthesis protein B (DnaB), which unwinds parental DNA by encircling and translocating on the lagging strand template. Each DnaB helicase binds to at least two molecules of DNA polymerase III (Pol III), which synthesize the leading and the lagging strands in association with the processivity clamp-β. The two replication forks emanating from oriC travel around the chromosome in opposite directions at a rate of ~60 kb per minute and terminate in a specialized region across from the origin. This termination zone contains 10 *ter* sites (A–J), which can bind the DNA replication terminus site-binding protein (Tus) to form potent and polar replication fork barriers (reviewed in REF. 11) (FIG. 2A). The *ter* sites are oriented such that the leftward fork can pass the first five *ter* sites that it encounters but stalls at the next five sites. Conversely, the rightward fork passes through the *ter* sites at which the leftward fork is stalled but stalls at the sites that the leftward fork passes. In this way, forks can enter but cannot leave the termination zone.

The function of the Tus–*ter* complexes in replication termination remains unclear. It has been debated[11] whether fork encounter occurs after one of the two forks has already stalled at a *ter* site (FIG. 2Ba), or whether fork encounter occurs between two *ter* sites (FIG. 2Bb). Some forks clearly collide with the non-permissive face of a Tus–*ter* complex, as shown by 2D gel electrophoresis[12].

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

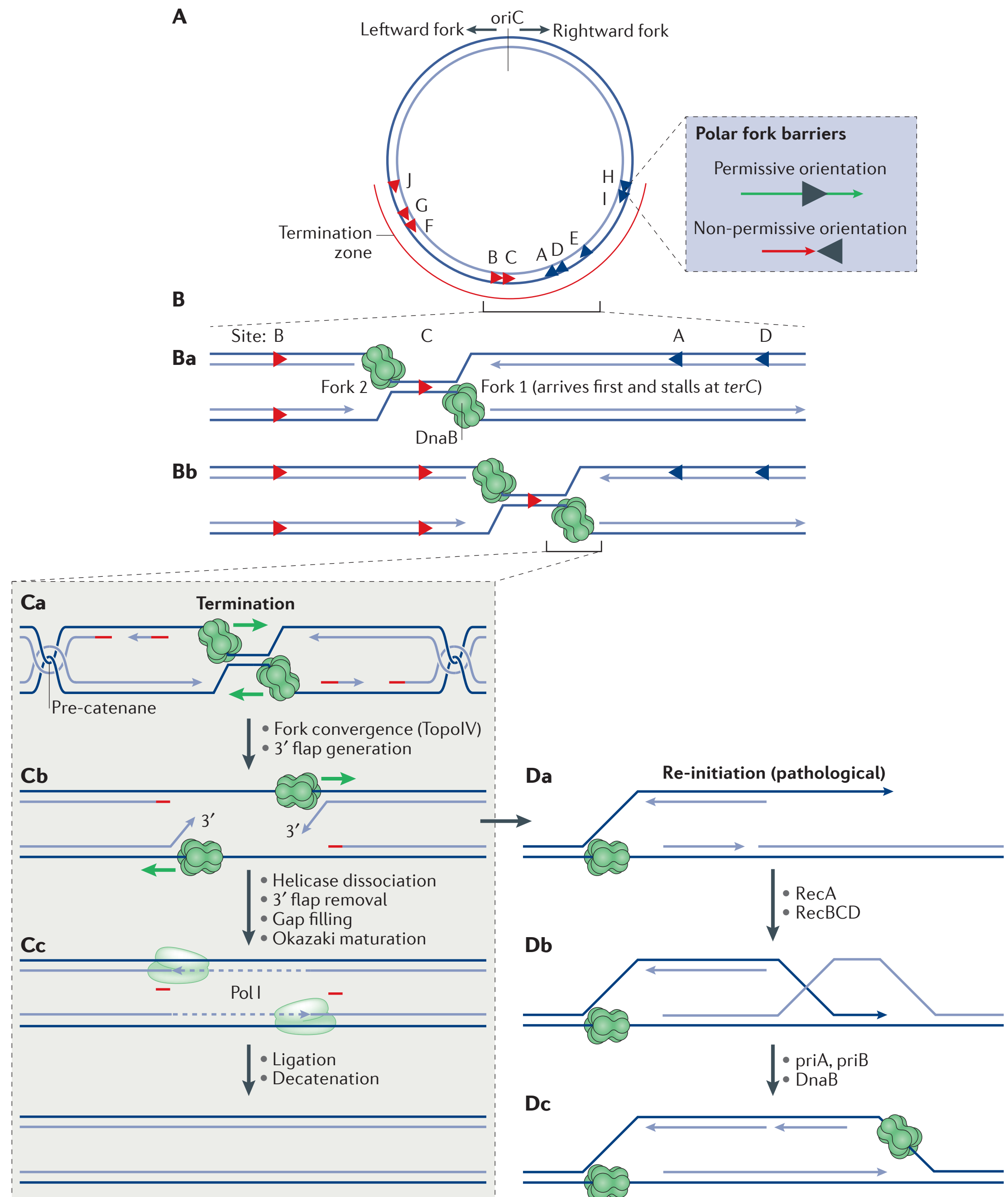


Figure 2 | **Replication termination in *Escherichia coli*. A** | Depiction of the *Escherichia coli* chromosome, including the origin of replication oriC, and the ten *ter* sites (A–J) shown as red and blue arrowheads. The termination zone is underlined in red. The *ter* sites are oriented such that the leftward fork can pass the first five *ter* sites that it encounters (red arrowheads), but stalls at the next five sites. Conversely, the rightward fork passes through the *ter* sites marked as blue arrowheads and stalls at the following sites. In the box, the green arrow represents a replication fork passing through a *ter* site in the permissive orientation, and the red arrow represents a fork stalling at a *ter* site in the non-permissive orientation. **B** | Two scenarios of fork stalling in the termination zone. **Ba** | The rightward fork (fork 1) arrives first and stalls at *terC*, followed by the arrival of the leftward fork (fork 2). **Bb** | The two forks arrive at the termination zone simultaneously and meet between *terC* and *terA*. **C** | Possible mechanism of *E. coli* replication termination. **Ca** | The forks converge between ter sites with the formation of pre-catenanes. **Cb** | Two DNA synthesis protein B (DnaB) replicative helicase complexes pass each other and collide with the downstream leading strand, generating a 3′ flap. DnaB dissociates, the 3′ flap is removed, the gaps are filled and the final Okazaki fragment is processed by DNA polymerase I (Pol I). **Cc** | Nicks are ligated and the final catenane, which is generated during the completion of DNA synthesis, is removed (not shown). **D** | Possible mechanism of replication re-initiation. **Da** | If the 3′ flaps are not removed or remodelled, a new replication fork is established, which prevents the completion of replication termination. **Db** | The free end re-invades the sister chromatid using recombination protein A (RecA) and RecBCD, which establish a new replication fork. **Dc** | The Holliday junction is resolved and DnaB is re-loaded onto the fork by primosomal protein A (priA) and priB. TopoIV, topoisomerase IV.

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

However, when the *tus* gene is deleted (Δ*tus*), the location of most fork fusions remains approximately 10,000 bp to the right of *terC*[13,14]. Therefore, most forks seem to converge between *ter* sites C and A (FIG. 2Bb). Surprisingly, Δ*tus* strains have no abnormal growth phenotypes[15], suggesting that the Tus–*ter* system is not an integral part of the termination machinery, but that instead it has other roles (see below).

How do the five steps of replication termination (outlined in FIG. 1) unfold between *ter* sites? The two type II topoisomerases in *E. coli* are DNA gyrase and topoisomerase IV (TopoIV). Consistent with their substrate specificities *in vitro*, gyrase relaxes positive supercoils ahead of the fork during the elongation stage of DNA replication, whereas TopoIV is required for the decatenation of the fully replicated daughter molecules[16] (FIG. 2Ca,Cb). Evidence also indicates that TopoIV is required to resolve pre-catenanes and to thereby enable fork convergence[17,18]. Whether converging forks clash during encounter is unknown. A recently suggested model for gap filling is based on the observation that, in cells lacking 3′ flap removal activity, replication re-initiates, as shown by deep sequencing of genomic DNA[14]. This DNA amplification in the termination zone is suppressed by the absence of primosomal protein A (priA), which promotes oriC-independent loading of DnaB. Together with other reports[19,20], these data imply that, when replication forks meet in wild-type cells, a 3′ flap is generated (FIG. 2Cb). This flap is normally degraded or remodelled and the gap is subsequently filled (FIG. 2Cc). Pol I may use its 5′ to 3′ exonuclease activity to remove the RNA primer of the last Okazaki fragment[21], as seen during replication elongation, to facilitate ligation (FIG. 2Cc). If the flap is not removed, two replication forks are established following strand invasion and priA-dependent loading of DnaB (FIG. 2D). Notably, the extent of re-replication is greatly increased in the absence of Tus[14,19,21]. Therefore, it seems that the primary function of Tus is not to promote site-specific termination, but rather to limit the extent of any re-replication after aberrant initiation in the termination zone.

To validate this model of replication termination, it will be essential to determine whether the 3′ flap is generated in unperturbed cells. If so, how does this occur? It has been proposed that when DnaB reaches the 3′ end of the leading strand of the opposing fork, it unwinds this strand (FIG. 2Cb). However, biochemical studies indicate that, in this situation, DnaB would pass over the 3′ end and would keep translocating along double-stranded DNA (dsDNA) without further DNA unwinding[22]. Therefore, either DnaB behaves differently as part of the replisome, or the flap is generated by another DNA helicase. A related issue concerns replisome disassembly. Does the encounter of two Pol III holoenzymes, in which the leading and the lagging strand polymerases are physically coupled to DnaB, cause a steric clash that requires replisome disassembly before gaps can be filled? The model suggesting that DnaB creates a 3′ flap implies that DnaB is unloaded late in replication termination, after the DnaB molecules of converging forks have passed each other. In this view, the polymerases would need to either be unloaded or disengage from the leading strands to allow converging DnaB complexes to pass each other (FIG. 2Cb). Further work is needed to address these issues, including to elucidate why *E. coli* termination seems to be so susceptible to re-initiation.

Pre-catenanes
A double-stranded intertwine between two DNA molecules that occurs behind the replication fork, on recently replicated DNA.

Replisomes
The collections of proteins involved in DNA replication at the replication fork.

Fork stalling
A pathological situation in which replication fork progression is impaired.

Okazaki fragment
A short DNA fragment synthesized on the lagging strand template.

Catenane
A double-stranded intertwine between two DNA molecules.

Replication termination in simian virus 40

Termination of replication has been extensively studied in the context of the mammalian DNA tumour virus SV40, the small circular (plasmid) chromosome of which comprises 5,200 bp (FIG. 3A). SV40 encodes its own replicative helicase, large T antigen (T-ag), which cooperates with mammalian host replication factors to replicate the SV40 chromosome[23]. Two replication forks are established at the origin and terminate on the opposite side of the plasmid (FIG. 3A). Relocation of the origin leads to a corresponding shift in the termination zone[24], indicating that SV40 lacks genetically encoded termination sites.

Two long-lived intermediates have been detected during SV40 replication termination. The first intermediate is a late theta structure[25–28], in which all but the final ~450 bp of the SV40 chromosome is replicated[29] (FIG. 3Ba). The accumulation of this intermediate might be explained if, during fork convergence, the removal of supercoils ahead of the fork becomes inefficient, and kinetically slower formation of pre-catenanes takes over (FIG. 3Bb). In cell-free extracts, replicated SV40 plasmid dimers contain 5–20 catenanes[30,31] (FIG. 3Bc), which is consistent with the formation of pre-catenanes during fork convergence (FIG. 3Bb). Catenated plasmid dimers are ultimately resolved into circular monomers (FIG. 3Bd). The final stage of SV40 DNA synthesis requires topoisomerase II (TopoII)[32,33], indicating that the removal of pre-catenanes allows convergence, as seen in bacteria. The second long-lasting intermediate is a single-stranded DNA (ssDNA) gap of ~60 nucleotides, which is observed in the termination region after forks converge[34] (FIG. 3C), although the cause of this gap is a matter of speculation (see below). Importantly, gap filling and decatenation seem to be mechanistically independent events[30].

Many concepts in SV40 termination are linked to models of how T-ag functions. Early results suggested that T-ag dissociates from DNA when replication is only 80% complete, possibly at the onset of convergence[35]. However, such a model does not explain how the final 20% of parental DNA is unwound, and it is incompatible with evidence that complete SV40 DNA replication can be reconstituted in a defined system in which T-ag is the only DNA helicase[36]. An early model of DNA unwinding by T-ag proposed that it encircles the DNA duplex and translocates along it[37]. In this view, T-ag molecules would stall at encounter and would need to be disassembled before gap filling. However, more recent work indicates that T-ag, like other replicative DNA helicases, translocates along one strand of the DNA[38] (FIG. 3Ca), suggesting that converging T-ag molecules should be able to pass each other (FIG. 3Cb,Cc). T-Ag stalling at the downstream Okazaki fragment might inhibit ligation and could explain the persistence of the 60 nucleotide ssDNA after decatenation[34]. To understand SV40 termination more fully, it will be crucial to determine exactly when T-ag dissociates from DNA, whether this is an active process, and the

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

consequences of disrupting its unloading. In summary, SV40 replication termination involves at least two long-lived intermediates (late theta structures and gapped molecules), and future studies will be required to address how these intermediates are linked to replisome disassembly.

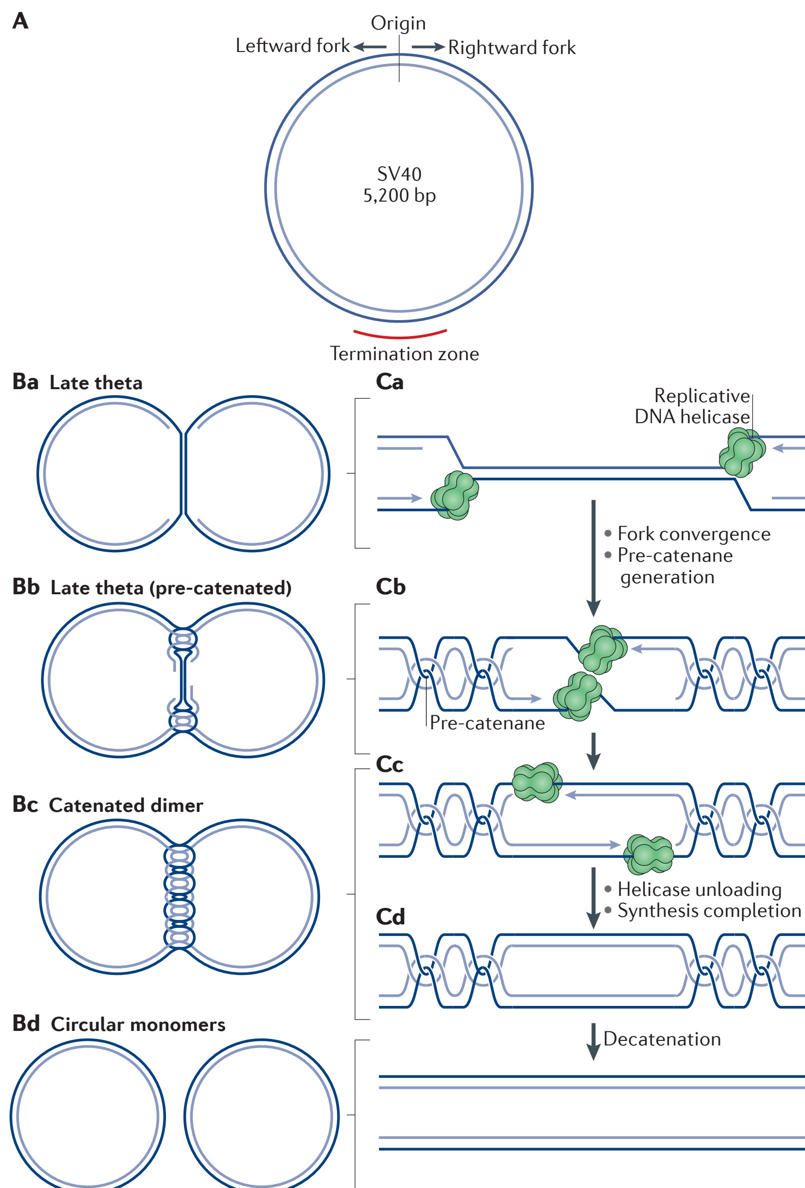


Figure 3 | **Model for simian virus 40 DNA replication termination. A** | The simian virus 40 (SV40) chromosome is a plasmid that includes the origin of replication and the termination zone (underlined in red). **B** | Late stages of SV40 DNA replication. **Ba** | Late theta intermediate, when fork convergence begins. **Bb** | Fork convergence, when superhelical stress is dissipated by the formation of pre-catenanes. **Bc** | Catenated dimers are generated when pre-catenanes are converted into catenanes at the end of replication. **Bd** | Decatenation produces two circular monomers. **C** | Hypothetical mechanism of SV40 replication termination. **Ca** | Forks stall when they come within 450 bp of each other, possibly owing to the reduced formation of pre-catenanes. **Cb** | Forks converge, leading to the formation of pre-catenanes and the encounter of the large T antigen (T-ag) helicases. Whether the helicases stall upon encounter is not known. **Cc** | The helicases pass each other and stall at the downstream Okazaki fragment, accounting for the single-stranded DNA gap that persists on replicated molecules. T-Ag is unloaded, the remaining gaps are filled. **Cd** | The catenanes are removed, yielding fully replicated and decatenated daughter chromosomes.

Replication termination in eukaryotes

The mechanism of replication in eukaryotic cells is complex[3,4,37,39] (BOX 1). The process begins in the G1 phase of the cell cycle when six minichromosome maintenance ATPases (MCM2–MCM7), which together form the MCM2–7 replicative DNA helicase motor, are recruited to each origin of replication. In S phase, MCM2–7 is converted into the active CMG helicase, which is composed of cell division cycle 45 (CDC45), MCM2–7 and the four-subunit complex Go-Ichi-Ni-San (GINS). CMG unwinds the origin, and this is followed by the assembly of two replisomes that copy the DNA using distinct leading and lagging strand DNA polymerases. Budding yeast replication was recently reconstituted *in vitro* using purified components[40–42]. This system supports rapid initiation and elongation, although termination is inefficient[43]. Given that termination is supported by frog egg extracts[44,45], it is likely that the yeast reconstituted system is missing one or more termination proteins. Below, we discuss recent insights into the processes that underlie eukaryotic replication termination, including the active disassembly of the replisome.

Genomic distribution of termination sites. First, we consider where on eukaryotic chromosomes termination events occur. Using a fork synchronization protocol, ~70 of the ~300 termination events mapped in budding yeast reproducibly occurred in the same chromosomal location, possibly owing to genetically encoded elements[46]. More recently, Okazaki fragments have been mapped genome-wide in unsynchronized budding yeast cells to identify fork merger zones[47]. This analysis showed that termination events generally occur midway between origins and that the more active the two origins, the better defined the termination zone between them. Alteration of origin firing (activation) caused predictable changes in termination zones, which was consistent with the large majority of termination events being sequence nonspecific and mostly dictated by initiation patterns[47,48]. Similar conclusions were reached from Okazaki fragment mapping in mammalian cells[49]. The absence of specific termination sites is well suited to dealing with the substantial level of stochasticity observed in eukaryotic origin firing. Only in rare instances are termination events site specific (see below).

Replication fork convergence. The SV40 termination model suggests that DNA synthesis slows during fork convergence, and studies in both SV40 and *E. coli* suggested that fork convergence requires the activity of TopoII. To study eukaryotic replication termination in frog egg extracts, forks were temporarily stalled at the outer edges of a ~500 bp array of LacI molecules, followed by isopropyl-β-D-thiogalactoside addition to induce locus-specific and synchronous termination events[44]. No slowing in the rate of DNA synthesis was observed during the synthesis of the final 500 bp of DNA. Although some topological stress may have dissipated before the disruption of the replication barrier, the completion of replication required the removal of ~50 supercoils. These results suggest that, in this cell-free system, the resolution

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

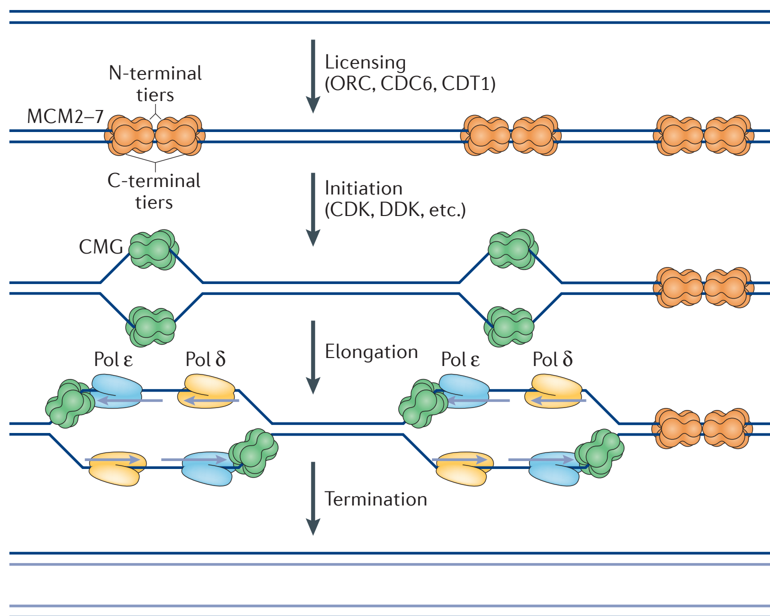
Box 1 | **Eukaryotic replication initiation and elongation: the basics**

We provide below a brief summary of eukaryotic DNA replication initiation and elongation (reviewed in REFS 3,4,37,39). Licensing of DNA replication occurs in the G1 phase of the cell cycle, when the origin recognition complex (ORC), the ATPase cell division cycle 6 (CDC6) and CDC10-dependent transcript 1 (CDT1) cooperate to recruit two minichromosome maintenance 2–7 (MCM2–7) complexes to each origin of replication, thereby forming the pre-replicative complex (pre-RC) (see the figure). MCM2–7 is a heterohexamer that is composed of the related ATPases MCM2 and MCM7 and serves as the motor of the replicative helicase. Within pre-RCs, two inactive MCM2–7 complexes encircle double-stranded DNA, with their amino-terminal tiers oriented towards each other to form a tight dimer interface.

In S phase, a subset of pre-RCs undergo activation by cyclin-dependent kinase (CDK), DBF4-dependent kinase (DDK) and many accessory factors, leading to the binding of two helicase cofactors, CDC45 and the four-subunit Go-Ichi-Ni-San (GINS) complex, to each MCM2–7 complex, thereby forming the active CDC45–MCM–GINS (CMG) helicase (see the figure). CMG encircles the leading strand and translocates along it in the 3′ to 5′ direction. Recent data suggest that the carboxy-terminal lobe of MCM2–7, which contains the ATPase motors of the helicase, forms the trailing edge of CMG[91]. CMG unwinds the origin, allowing the assembly of two DNA replication forks that travel away from the origin. Cells prevent re-replication by blocking licensing in S phase[1].

In yeast, although origin sequences are well-defined, initiation is partly stochastic, so that the programme of origin firing is probably unique in every cell. In higher eukaryotes, the DNA sequences of origins are poorly defined and initiation is inefficient and frequently occurs in large zones, resulting in replication programmes that are even more stochastic than in yeast.

The replisome is a macromolecular assembly that is composed of multiple protein complexes. The leading strand is synthesized continuously by DNA polymerase ε (Pol ε), whereas the lagging strand is composed of Okazaki fragments and is synthesized by Pol δ[92] (see the figure). Pol δ acquires processivity by its association with the ring-shaped protein proliferating cell nuclear antigen, which is deposited around DNA by replication factor C. The leading strand and every Okazaki fragment are primed by Pol α-primase, which synthesizes an approximately 10-nucleotide RNA primer and then extends it by 20–30 nucleotides of DNA before the switch to the more processive Pol ε or Pol δ occurs. When the 3′ end of one Okazaki fragment reaches the 5′ end of another Okazaki fragment, Pol δ carries out strand displacement synthesis (FIG. 4d). The resulting flap is removed by flap endonuclease 1. Long flaps are degraded by the helicase-nuclease DNA synthesis defective protein 2. The replisome also contains topoisomerase I, chromatin remodelling factors, checkpoint signalling proteins and cohesion establishment factors. CMG binds directly to Pol ε and indirectly to Pol α through chromosome transmission fidelity protein 4. Therefore, unlike in bacteria, leading and lagging strand polymerases do not seem to form a stable complex in eukaryotes.



of topological stress is not rate-limiting for fork convergence, presumably owing to the efficient formation of pre-catenanes (FIG. 4a). Accordingly, DNA catenation was detected immediately following fork convergence[44], as previously reported[50,51]. Replicon size does not affect the number of catenanes formed[52], suggesting that pre-catenanes are primarily formed during replication termination rather than during elongation[9,50]. Strikingly, unlike observations in *E. coli* and SV40, fork convergence and synthesis completion do not require TopoII in egg extracts or yeast[44,53,54]. In the absence of TopoII, fully replicated daughter plasmids are generated that are highly catenated, indicating that the resolution of pre-catenanes is not essential for convergence in eukaryotic systems. In the future, it will be important to understand the differential requirements for type II topoisomerases in eukaryotic systems and in bacteria.

Replisome encounter. What happens when converging CMG complexes meet? In frog egg extracts, nascent leading strands pass each other without detectable pausing, followed by the rapid ligation of all nascent strands (FIG. 4), which indicates that, during fork encounter, there is either no steric clash or a very short-lived steric clash. This is consistent with our current understanding of replisome architecture. First, replisome-associated CMG seems to interact primarily with the leading strand template[55]. Therefore, the converging CMG complexes will encounter each other on different strands (FIG. 4a), thereby facilitating bypass. Second, unlike in *E. coli*, in eukaryotic cells, there is little evidence for the existence of a stable complex between the leading strand replication machinery (that is, CMG, Pol ε, and so on) and the lagging strand replication machinery (that is, Pol δ, proliferating cell nuclear antigen (PCNA), flap endonuclease 1 (FEN1), DNA ligase, and so on), although Pol α-primase (Pol α) does bind weakly to a CMG complex in yeast[56]. Such a separation between the leading and the lagging strand machineries allows the CMG of one fork to pass unobstructed onto the lagging strand template of the converging fork. The absence of pausing during encounter is attractive, as any instances of fork stalling are likely to be deleterious to genome stability. Curiously, when CMG encounters a covalent DNA–protein (~40 kDa) complex on the lagging strand template it stalls for a few minutes[57]. This observation raises the interesting possibility of specific evolutionary adaptations that prevent a clash during the encounter of two CMGs during termination.

Replisome disassembly. The removal of the CMG helicase from chromatin is emerging as a key event in eukaryotic replication termination. Late in the S phase of budding yeast and frogs, K48-linked ubiquitin chains are assembled on MCM7 (REFS 45,58) (FIG. 4e). In budding yeast, MCM7 polyubiquitylation and CMG dissociation both require the E3 ubiquitin ligase SCF^{Dia2} (Skp, Cullin, F-box-containing complex associated with Dia2)[58], strongly suggesting that MCM7 ubiquitylation is causally linked to CMG unloading. Recently, $CRL2^{LRR1}$ (Cullin RING ligase 2 associated with LRR1) was identified as the ubiquitin ligase that promotes MCM7 ubiquitylation

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

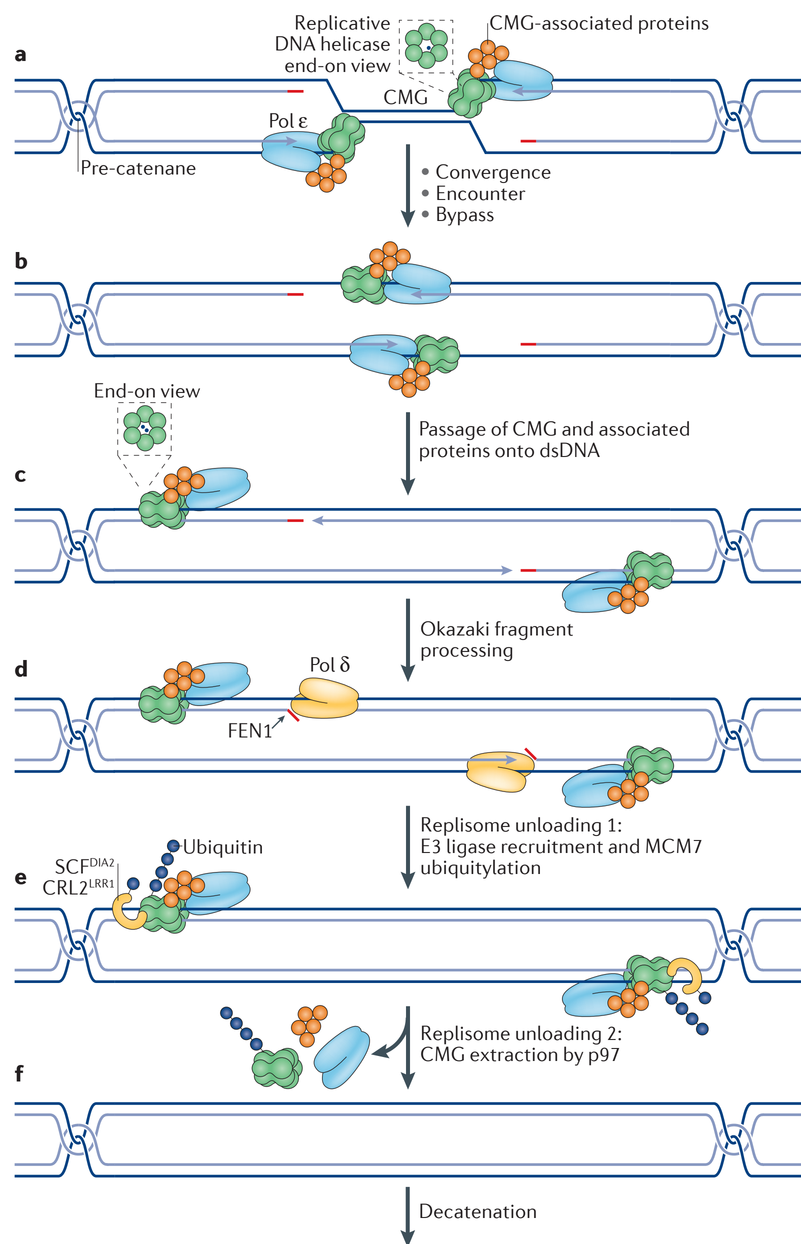


Figure 4 | **Model of eukaryotic replication termination. a** | After copying most of the replicon, forks come too close to each other to allow the formation of supercoils in the unreplicated DNA, leading to the onset of convergence. During convergence, which lasts until forks encounter each other, topological stress is relieved by the formation of pre-catenanes. An end-on view of CMG (CDC45–MCM–GINS) illustrates the presence of single-stranded DNA (ssDNA) in its central channel. **b** | The encounter causes no detectable fork stalling, implying that converging CMGs bypass each other. **c** | After bypass, CMG helicases keep translocating until they pass over the ssDNA–double-stranded DNA (dsDNA) junction of the downstream Okazaki fragment, presumably by translocating along dsDNA (see end-on view). This allows leading strands to be extended to the downstream Okazaki fragments. **d** | The final Okazaki fragment is processed, possibly by *de novo* recruitment of DNA polymerase δ (Pol δ) and by 3′ flap processing by flap endonuclease 1 (FEN1). **e** | Once CMG encircles dsDNA, it undergoes polyubiquitylation on its MCM7 subunit by SCF^{DIA2} (Skp, Cullin, F-box-containing complex associated with DIA2) or $CRL2^{LRR1}$ (Cullin RING ligase 2 associated with LRR1). The ubiquitylated MCM7 is extracted from chromatin by the ATPase p97 (not shown). **f** | Catenanes are removed.

and CMG unloading at the end of S phase in worms and frogs[59,60]. SCF^{Dia2} may be constitutively associated with replisomes in yeast[61], whereas frog $CRL2^{LRR1}$ only binds to terminating replisomes[59]. Once CMG is ubiquitylated, it is removed from chromatin by the ATPase p97 (also known as VCP)[45,58,62] (FIG. 4e,f), which cooperates with diverse cofactors to extract ubiquitylated proteins from their local environments[63]. Data from frogs indicate that ubiquitylated MCM7 is not degraded by the proteasome after chromatin extraction, suggesting that ubiquitylated CMG is recycled after disassembly[64]. Yeast cells lacking Dia2 exhibit constitutive activation of the replication checkpoint, sensitivity to DNA-damaging agents and gross chromosomal rearrangements[65–67]. In the absence of $CRL2^{LRR1}$, worm CMG persists on chromatin until prophase, when it is unloaded by a second pathway that involves p97 and UBX domain-containing 3 (UBXN3; the worm orthologue of human FAS-associated factor 1 (FAF1))[60]. Importantly, combined knockdown of LRR1 and UBXN3 stabilizes CMG on chromatin until metaphase and is synthetically lethal, suggesting that replisome unloading is essential for viability[60]. Whether vertebrates also have a mitotic CMG unloading mechanism is unknown. It will also be of great interest to elucidate the consequences of defective CMG unloading and to determine whether these defective unloading consequences underlie any human diseases.

Interestingly, CMG is unloaded only after the gap between the leading strand of one fork and the lagging strand of the converging fork has been filled and ligated[44] (FIG. 4d–f). As such, CMG unloading is one of the latest known events in eukaryotic replication termination, occurring once CMG is associated with dsDNA. Converging CMGs probably pass each other and keep translocating along the leading strand template until they reach the downstream Okazaki fragment, whereupon they pass onto dsDNA (FIG. 4c). This model is consistent with the observation that, when purified CMG reaches a ssDNA–dsDNA junction, it passes over the junction and keeps moving along dsDNA without further DNA unwinding, which requires a flap, as has also been reported for DnaB[68,69]. It will be important to examine whether CMG can also pass over a junction containing an RNA–DNA hybrid, as would be the case for an Okazaki fragment. Together, these data suggest that MCM7 ubiquitylation occurs when CMG encircles dsDNA. Importantly, synthesis completion is unaffected when CMG unloading is inhibited, which is consistent with unloading occurring as a later event in termination[44,59].

A crucial unresolved issue concerns the trigger for CMG unloading. Given that MCM2–7 loading (and therefore CMG assembly) in S phase is prohibited in order to prevent re-replication[1], premature CMG removal from active forks must be prevented to avoid fork stalling and breakage. An attractive model suggests that the presence of dsDNA in the central channel of CMG leads to the recruitment of $CRL2^{LRR1}$ (or, in yeast, this leads to the activation of already bound SCF^{Dia2}) owing to a conformational change in CMG. Remarkably, in yeast the induction of SCF^{Dia2} expression in G1 phase can unload CMG complexes that remained on the chromatin from

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

the previous S phase[58], suggesting that SCF^{Dia2} can target CMG that is associated with dsDNA. The advantage of this mechanism is that it cannot operate on active replication forks where CMG encircles ssDNA[55] (BOX 1). To discriminate between CMG complexes during termination and licensed MCM2–7 complexes, which also encircle dsDNA, the ligase might detect the presence of CDC45 and/or GINS, or that it might be inhibited by the dimerization of licensed MCM2–7 complexes. Alternatively, the MCM7 ubiquitin ligase might detect the juxtaposition of converged CMG complexes or the collision of CMG with the rear face of PCNA molecules from the converging fork. Interestingly, CMG complexes are also actively unloaded when forks converge on a DNA interstrand crosslink (ICL)[55,70]. Although this pathway involves MCM7 ubiquitylation and p97 (REF. 64), it operates on CMG complexes that encircle ssDNA on either side of an ICL and, unlike termination-dependent CMG unloading, it requires BRCA1 bound to BRCA1-associated RING domain protein 1 (BARD1)[64,70]. Therefore, the mechanisms of CMG unloading during termination and ICL repair are clearly distinct.

How does the remainder of the replisome dissociate from chromatin during termination? CMG makes direct or indirect contact with numerous proteins at the replication fork, including the components of the replisome progression complex and Pol ε[39,61,71]. Therefore, it is likely that numerous replication proteins are passively unloaded as an indirect consequence of CMG unloading. Consistent with this idea, CMG and Pol ε dissociate with similar kinetics[44], and blocking CMG unloading leads to the retention of most RPC components at the fork, including Pol ε[59]. To determine whether SCF^{DIA2} or CRL2^{LRR1} target other replisome components, it will be important to test whether the loss of these E3 ligases mimics the elimination of ubiquitylation sites on MCM7. Proteins that do not interact with CMG are probably removed from chromatin independently of replication termination. For example, PCNA is continually unloaded by ATPase family AAA domain-containing protein 5 (ATAD5; also known as ELG1)[72,73], which should also lead to the removal of PCNA-interacting proteins, such as FEN1, DNA ligase and Pol δ at the lagging strand maturation machinery.

Gap filling. In frog egg extracts, leading strands are extended past each other without visible pausing until they come within a few nucleotides of the downstream Okazaki fragment, upon which the two strands are rapidly ligated[44] (in contrast to the situation in SV40 DNA in which gaps persist). This observation implies that neither the resolution of topological stress nor CMG unloading is rate-limiting for synthesis completion. Indeed, by passing over the downstream Okazaki fragment, CMG would vacate the ssDNA–dsDNA junction and make room for the enzymes that carry out Okazaki fragment processing. CMG may also drag Pol ε, with which it forms a stable complex[71], away from the junction[59]. This is potentially advantageous because, unlike Pol δ, Pol ε does not functionally cooperate with FEN1 for Okazaki fragment processing[74]. Thus, the removal of Pol ε should make room for Pol δ and should facilitate processing. However, this mechanism does have a potential disadvantage. If CMG is able to translocate a considerable distance along dsDNA before being unloaded, re-replication would be promoted if CMG encounters a downstream Okazaki fragment with a 5′ flap, which is analogous to the situation in *E. coli* (FIG. 2Da). In agreement with this possibility, loss of CRL2^{LRR1} in worms seems to induce re-replication[75]. In the future, it will be crucial to assess whether short stretches of re-replication normally occur in healthy cells or in cells in which a component of the Okazaki maturation machinery, such as FEN1, has been compromised.

Decatenation of replicated chromosomes. When the last turn of the parental DNA duplex is unwound, the daughter molecules are catenated through one interwinding (FIG. 1f). In addition, any pre-catenanes are automatically converted into catenanes[9]. The resolution of catenanes is unlikely to be mechanistically distinct from the unlinking of pre-catenanes behind replication forks[18]. We note that both *E. coli* and yeast can carry out a mechanistically distinct decatenation process, which is driven by the supercoiling of the chromosome[76,77]. The mechanism described in yeast requires condensin loading and spindle formation[77], which only occur during mitosis. Therefore, it is unlikely to be extensively used during replication termination, which occurs during S phase.

Site-specific replication termination. Although most eukaryotic termination events seem to be sequence nonspecific (see above), at least two classes of site-specific termination exist[78,79]. The first class is caused by sequence-specific replication fork barriers (RFBs) that stall one fork long enough for a converging fork to arrive. The best-characterized example is the polar RFB in the ribosomal DNA (rDNA) locus, which contains tandem repeats of highly transcribed ribosomal gene clusters. Each rDNA cluster contains an RFB comprising a termination element (*Ter* in budding yeast) that is bound by a terminator protein (fork blocking protein 1 (Fob1) in budding yeast). Similar to the Tus–*ter* complex in *E. coli*, the Fob1–*Ter* complex creates a polar RFB and thus prevents head-on collisions between the replication and the transcription machineries, which have the potential to cause genome instability[80]. Although the mechanism of fork arrest at the rDNA RFB is fairly well defined[81,82], how termination unfolds at this locus, or any other RFB, has not been examined. In frog extracts, stalled replication forks can readily restart and terminate replication[44]. Therefore, it is likely that the replisome from the fork that is stalled at the rDNA RFB can restart once the converging fork displaces the terminator protein. The replisomes probably then pass each other and terminate replication using the same mechanism as used at other loci (FIG. 4). In budding yeast, the helicase rDNA recombination mutation protein 3 (Rrm3) is required for the progression of replication forks past protein–DNA complexes. Interestingly, although *rrm3Δ* strains exhibit an approximately twofold increase in replication fork stalling at Fob1–*Ter*, they show an approximately tenfold accumulation of converged forks at this RFB, suggesting a

Replicon
A DNA region that is replicated by two replication forks emanating from a single origin.

Replisome progression complex
A large assembly of proteins bound directly or indirectly to the replicative CMG (CDC45–MCM–GINS) helicase.

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

specific role for Rrm3 in fork convergence[83]. Importantly, *rrm3Δ* strains exhibit no genome-wide defects in replisome disassembly[58], suggesting a specialized role for Rrm3 at the rDNA RFB, perhaps in displacing Fob1 (REF. 81). In the future, it will be interesting to determine whether the mechanism of termination at site-specific RFBs differs in any fundamental way from the general mechanism that occurs at most genomic termination sites. The mechanism of fork stalling at RFBs, as well as their roles in other processes, such as gene silencing, imprinting and ageing, are not discussed in this Review, as they have been reviewed elsewhere[78,79].

The second class of site-specific termination events occurs at telomeres[84]. At telomeres, replication ceases when the fork reaches the end of the chromosome. Importantly, when the fork copies the telomere, the leading strand seems to be extended to within a few nucleotides of the chromosome end[85–87], similar to the run-off DNA synthesis carried out by purified Pol ε[88]. Together, these observations suggest that CMG slides off the end of the leading strand template, allowing Pol ε to reach the end of the chromosome[89]. Given the evidence that Pol α is tethered to the replisome through CMG[56,59], Pol α should dissociate with CMG, thereby preventing new priming. This might explain why the amount of DNA that is lost on the lagging strand in humans is approximately equivalent to the size of an Okazaki fragment[86]. The above model predicts that replisome dissociation at telomeres does not require MCM7 ubiquitylation, and it will be important to test this model in the future.

Outlook

In light of the recently acquired insights into the mechanism of eukaryotic replication termination discussed above, it will be fascinating to revisit termination mechanisms in bacteria and viruses and to determine the similarities and differences between these mechanisms. On the basis of studies in viruses and bacteria, termination is a challenging process that unfolds in fits and starts. By contrast, in eukaryotic cells, neither precatenane resolution nor helicase unloading is essential for the completion of DNA synthesis, indicating that termination is a highly robust process.

The identification of an active CMG removal pathway in eukaryotes provides the first hint that the endgame of DNA replication might be as highly regulated as initiation, albeit by ubiquitylation rather than by the phospho-regulatory mechanisms that are used during replication initiation. An important question is whether bacterial and viral systems involve an active helicase removal mechanism. If not, is this because the helicase is less tightly clamped around DNA, allowing passive dissociation? Passive dissociation is also likely to occur during replication elongation, which might explain the existence of helicase-reloading pathways in bacteria but not in eukaryotes[90]. Furthermore, in eukaryotes, the lack of interference between converging CMG complexes prevents the persistence of DNA gaps at the end of replication. By comparison, do converging DnaB molecules pass each other during termination? If not, and if they stall upon contact, is this due to the physical coupling between the leading and the lagging strand polymerases?

Another important challenge is to determine whether replication termination is as susceptible to re-initiation in eukaryotes as it is in bacteria. If so, does deregulation of termination contribute to genomic instability and human disease? How do the triggers for CMG unloading during termination and ICL repair differ? Finally, what effect does chromatin structure have on the mechanism of termination? Can a CMG that has terminated replication and is translocating on dsDNA displace nucleosomes? Answering these questions will be important to deepen our understanding of a neglected but crucial part of the DNA replication process.

1. Siddiqui, K., On, K. F. & Diffley, J. F. Regulating DNA replication in eukarya. *Cold Spring Harb. Perspect. Biol.* **5**, a012930 (2013).
2. Skarstad, K. & Katayama, T. Regulating DNA replication in bacteria. *Cold Spring Harb. Perspect. Biol.* **5**, a012922 (2013).
3. Balakrishnan, L. & Bambara, R. A. Okazaki fragment metabolism. *Cold Spring Harb. Perspect. Biol.* **5**, a010173 (2013).
4. Costa, A., Hood, I. V. & Berger, J. M. Mechanisms for initiating cellular DNA replication. *Annu. Rev. Biochem.* **82**, 25–54 (2013).
5. Berezney, R., Dubey, D. D. & Huberman, J. A. Heterogeneity of eukaryotic replicons, replicon clusters, and replication foci. *Chromosoma* **108**, 471–484 (2000).
6. Zechiedrich, E. L. & Osheroff, N. Eukaryotic topoisomerases recognize nucleic acid topology by preferentially interacting with DNA crossovers. *EMBO J.* **9**, 4555–4562 (1990).
7. Champoux, J. J. & Been, M. D. in *Mechanistic Studies of DNA Replication and Genetic Recombination: ICN-UCLA Symposia on Molecular and Cellular Biology* (ed. Alberts, B.) 809–815 (New York Academic Press, 1980).
8. Postow, L., Crisona, N. J., Peter, B. J., Hardy, C. D. & Cozzarelli, N. R. Topological challenges to DNA replication: conformations at the fork. *Proc. Natl Acad. Sci. USA* **98**, 8219–8226 (2001).
9. Ullsperger, C., Vologodskii, A. & Cozzarelli, N. R. Unlinking of DNA by topoisomerases during DNA replication. *Nucleic Acids Mol. Biol.* **9**, 115–142 (1995).
10. Vafabakhsh, R. & Ha, T. Extreme bendability of DNA less than 100 base pairs long revealed by single-molecule cyclization. *Science* **337**, 1097–1101 (2012).
11. Dimude, J. U., Midgley-Smith, S. L., Stein, M. & Rudolph, C. J. Replication termination: containing fork fusion-mediated pathologies in *Escherichia coli*. *Genes (Basel)* **7**, 40 (2016).
12. Duggin, I. G. & Bell, S. D. Termination structures in the *Escherichia coli* chromosome replication fork trap. *J. Mol. Biol.* **387**, 532–539 (2009).
13. Ivanova, D. *et al.* Shaping the landscape of the *Escherichia coli* chromosome: replication–transcription encounters in cells with an ectopic replication origin. *Nucleic Acids Res.* **43**, 7865–7877 (2015).
14. Rudolph, C. J., Upton, A. L., Stockum, A., Nieduszynski, C. A. & Lloyd, R. G. Avoiding chromosome pathology when replication forks collide. *Nature* **500**, 608–611 (2013).
Provides strong evidence that defective termination in bacteria can lead to the formation of flap structures that stimulate the re-initiation of replication.
15. Duggin, I. G., Wake, R. G., Bell, S. D. & Hill, T. M. The replication fork trap and termination of chromosome replication. *Mol. Microbiol.* **70**, 1323–1333 (2008).
16. Levine, C., Hiasa, H. & Marians, K. J. DNA gyrase and topoisomerase IV: biochemical activities, physiological roles during chromosome replication, and drug sensitivities. *Biochim. Biophys. Acta* **1400**, 29–43 (1998).
17. Espeli, O., Levine, C., Hassing, H. & Marians, K. J. Temporal regulation of topoisomerase IV activity in *E. coli*. *Mol. Cell* **11**, 189–201 (2003).
18. Hiasa, H. & Marians, K. J. Two distinct modes of strand unlinking during theta-type DNA replication. *J. Biol. Chem.* **271**, 21529–21535 (1996).
Demonstrates the requirement for a type II topoisomerase during fork convergence in a bacterial reconstituted system.
19. Hiasa, H. & Marians, K. J. Tus prevents overreplication of oriC plasmid DNA. *J. Biol. Chem.* **269**, 26959–26968 (1994).
20. Wendel, B. M., Courcelle, C. T. & Courcelle, J. Completion of DNA replication in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **111**, 16454–16459 (2014).
21. Markovitz, A. A new *in vivo* termination function for DNA polymerase I of *Escherichia coli* K12. *Mol. Microbiol.* **55**, 1867–1882 (2005).
22. Kaplan, D. L. & O'Donnell, M. DnaB drives DNA branch migration and dislodges proteins while encircling two DNA strands. *Mol. Cell* **10**, 647–657 (2002).
23. Sowd, G. A. & Fanning, E. A wolf in sheep's clothing: SV40 co-opts host genome maintenance proteins to replicate viral DNA. *PLoS Pathog.* **8**, e1002994 (2012).
24. Weaver, D. T., Fields-Berry, S. C. & DePamphilis, M. L. The termination region for SV40 DNA replication directs the mode of separation for the two sibling molecules. *Cell* **41**, 565–575 (1985).
25. Seidman, M. M. & Salzman, N. P. Late replicative intermediates are accumulated during simian virus 40 DNA replication *in vivo* and *in vitro*. *J. Virol.* **30**, 600–609 (1979).
26. Tapper, D. P. & Depamphilis, M. L. Discontinuous DNA-replication: accumulation of Simian virus 40 DNA at specific stages in its replication. *J. Mol. Biol.* **120**, 401–422 (1978).

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

27. Sebring, E. D., Kelly, T. J. Jr, Thoren, M. M. & Salzman, N. P. Structure of replicating Simian virus 40 deoxyribonucleic acid molecules. *J. Virol.* **8**, 478–490 (1971).
28. Levine, A. J., Kang, H. S. & Billheimer, F. E. DNA replication in SV40 infected cells. I. Analysis of replicating SV40 DNA. *J. Mol. Biol.* **50**, 549–568 (1970).
29. Tapper, D. P. & DePamphilis, M. L. Preferred DNA sites are involved in the arrest and initiation of DNA synthesis during replication of SV40 DNA. *Cell* **22**, 97–108 (1980).
30. Sundin, O. & Varshavsky, A. Arrest of segregation leads to accumulation of highly intertwined catenated dimers: dissection of the final stages of SV40 DNA replication. *Cell* **25**, 659–669 (1981).
31. Sundin, O. & Varshavsky, A. Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers. *Cell* **21**, 103–114 (1980).
Proposes a mechanism for fork convergence that involves fork rotation and pre-catenane generation during SV40 replication termination.
32. Ishimi, Y., Sugasawa, K., Hanaoka, F., Eki, T. & Hurwitz, J. Topoisomerase II plays an essential role as a swivelase in the late stage of Sv40 chromosome-replication *in vitro*. *J. Biol. Chem.* **267**, 462–466 (1992).
33. Snapka, R. M., Powelson, M. A. & Strayer, J. M. Swiveling and decatenation of replicating Simian virus 40 genomes in vivo. *Mol. Cell. Biol.* **8**, 515–521 (1988).
34. Chen, M. C., Birkenmeier, E. & Salzman, N. P. Simian virus 40 DNA replication: characterization of gaps in the termination region. *J. Virol.* **17**, 614–621 (1976).
35. Tack, L. C. & DePamphilis, M. L. Analysis of Simian virus 40 chromosome–T-antigen complexes: T-antigen is preferentially associated with early replicating DNA intermediates. *J. Virol.* **48**, 281–295 (1983).
36. Waga, S. & Stillman, B. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. *Nature* **369**, 207–212 (1994).
37. Takahashi, T. S., Wigley, D. B. & Walter, J. C. Pumps, paradoxes and ploughshares: mechanism of the MCM2-7 DNA helicase. *Trends Biochem. Sci.* **30**, 437–444 (2005).
38. Yardimci, H. *et al.* Bypass of a protein barrier by a replicative DNA helicase. *Nature* **492**, 205–209 (2012).
39. Bell, S. P. & Labib, K. Chromosome duplication in *Saccharomyces cerevisiae*. *Genetics* **203**, 1027–1067 (2016).
40. Kurat, C. F., Yeeles, J. T., Patel, H., Early, A. & Diffley, J. F. Chromatin controls DNA replication origin selection, lagging-strand synthesis, and replication fork rates. *Mol. Cell* **65**, 117–130 (2017).
41. Yeeles, J. T., Deegan, T. D., Janska, A., Early, A. & Diffley, J. F. Regulated eukaryotic DNA replication origin firing with purified proteins. *Nature* **519**, 431–435 (2015).
42. Yeeles, J. T., Janska, A., Early, A. & Diffley, J. F. How the eukaryotic replisome achieves rapid and efficient DNA replication. *Mol. Cell* **65**, 105–116 (2017).
43. Devbhandari, S., Jiang, J., Kumar, C., Whitehouse, I. & Remus, D. Chromatin constrains the initiation and elongation of DNA replication. *Mol. Cell* **65**, 131–141 (2017).
Describes a reconstituted yeast system that fully supports replication initiation and elongation but that exhibits limited capacity for termination.
44. Dewar, J. M., Budzowska, M. & Walter, J. C. The mechanism of DNA replication termination in vertebrates. *Nature* **525**, 345–350 (2015).
Describes a biochemical mechanism for replication termination in frog egg extracts.
45. Moreno, S. P., Bailey, R., Campion, N., Herron, S. & Gambus, A. Polyubiquitylation drives replisome disassembly at the termination of DNA replication. *Science* **346**, 477–481 (2014).
46. Fachinetti, D. *et al.* Replication termination at eukaryotic chromosomes is mediated by Top2 and occurs at genomic loci containing pausing elements. *Mol. Cell* **39**, 595–605 (2010).
47. McGuffee, S. R., Smith, D. J. & Whitehouse, I. Quantitative, genome-wide analysis of eukaryotic replication initiation and termination. *Mol. Cell* **50**, 123–135 (2013).
Shows that localization of termination events is dictated by the timing and location of initiation events in yeast.
48. Greenfeder, S. A. & Newlon, C. S. A replication map of a 61-kb circular derivative of *Saccharomyces cerevisiae* chromosome III. *Mol. Biol. Cell* **3**, 999–1013 (1992).
49. Petryk, N. *et al.* Replication landscape of the human genome. *Nat. Commun.* **7**, 10208 (2016).
50. Lucas, I., Germe, T., Chevrier-Miller, M. & Hyrien, O. Topoisomerase II can unlink replicating DNA by precatenane removal. *EMBO J.* **20**, 6509–6519 (2001).
51. Charbin, A., Bouchoux, C. & Uhlmann, F. Condensin aids sister chromatid decatenation by topoisomerase II. *Nucleic Acids Res.* **42**, 340–348 (2014).
52. Schalbetter, S. A., Mansoubi, S., Chambers, A. L., Downs, J. A. & Baxter, J. Fork rotation and DNA precatenation are restricted during DNA replication to prevent chromosomal instability. *Proc. Natl Acad. Sci. USA* **112**, E4565–E4570 (2015).
53. Baxter, J. & Diffley, J. F. X. Topoisomerase II inactivation prevents the completion of DNA replication in budding yeast. *Mol. Cell* **30**, 790–802 (2008).
Shows that topoisomerase II is not required for replication fork convergence in yeast and outlines the consequences of decatenation defects.
54. Dinardo, S., Voelkel, K. & Sternglanz, R. DNA topoisomerase II mutant of *Saccharomyces cerevisiae* — topoisomerase II Is required for segregation of daughter molecules at the termination of DNA-Replication. *Proc. Natl Acad. Sci. USA* **81**, 2616–2620 (1984).
55. Fu, Y. V. *et al.* Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. *Cell* **146**, 931–941 (2011).
56. Gambus, A. *et al.* A key role for Ctf4 in coupling the MCM2-7 helicase to DNA polymerase alpha within the eukaryotic replisome. *EMBO J.* **28**, 2992–3004 (2009).
57. Duxin, J. P., Dewar, J. M., Yardimci, H. & Walter, J. C. Repair of a DNA–protein crosslink by replication-coupled proteolysis. *Cell* **159**, 346–357 (2014).
58. Maric, M., Maculins, T., De Piccoli, G. & Labib, K. Cdc48 and a ubiquitin ligase drive disassembly of the CMG helicase at the end of DNA replication. *Science* **346**, 1253596 (2014).
Identifies a multistep replicative helicase unloading pathway that requires SCF^{Dia2} and p97 in yeast.
59. Dewar, J. M., Low, E., Mann, M., Raschle, M. & Walter, J. C. CRL2-Lrr1 promotes unloading of the vertebrate replisome from chromatin during replication termination. *Gene Dev.* **31**, 275–290 (2017).
Demonstrates that the ubiquitin ligase $CRL2^{Lrr1}$ is recruited to the replisome during termination and promotes CMG unloading in frog egg extracts.
60. Sonneville, R. *et al.* CUL-2^{LRR-1} and UBXN-3 drive replisome disassembly during DNA replication termination and mitosis. *Nat. Cell Biol.* http://dx.doi.org/10.1038/ncb3500 (2017).
Identifies two distinct CMG unloading pathways in worms that operate in S phase and mitosis and require $CRL2^{LRR1}$ and UBXN3, respectively.
61. Gambus, A. *et al.* GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat. Cell Biol.* **8**, 358–366 (2006).
62. Franz, A. *et al.* CDC-48/p97 coordinates CDT-1 degradation with GINS chromatin dissociation to ensure faithful DNA replication. *Mol. Cell* **44**, 85–96 (2011).
63. Meyer, H., Bug, M. & Bremer, S. Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat. Cell Biol.* **14**, 117–123 (2012).
64. Fullbright, G., Rycenga, H. B., Gruber, J. D. & Long, D. T. p97 promotes a conserved mechanism of helicase unloading during DNA cross-link repair. *Mol. Cell. Biol.* **36**, 2983–2994 (2016).
65. Pan, X. *et al.* A DNA integrity network in the yeast *Saccharomyces cerevisiae*. *Cell* **124**, 1069–1081 (2006).
66. Koepp, D. M., Kile, A. C., Swaminathan, S. & Rodriguez-Rivera, V. The F-box protein Dia2 regulates DNA replication. *Mol. Biol. Cell* **17**, 1540–1548 (2006).
67. Blake, D. *et al.* The F-box protein Dia2 overcomes replication impedance to promote genome stability in *Saccharomyces cerevisiae*. *Genetics* **174**, 1709–1727 (2006).
68. Kang, Y. H. *et al.* Interaction between human Ctf4 and the Cdc45/Mcm2-7/GINS (CMG) replicative helicase. *Proc. Natl Acad. Sci. USA* **110**, 19760–19765 (2013).
69. Kaplan, D. L., Davey, M. J. & O'Donnell, M. Mcm4,6,7 uses a "pump in ring" mechanism to unwind DNA by steric exclusion and actively translocate along a duplex. *J. Biol. Chem.* **278**, 49171–49182 (2003).
70. Long, D. T., Joukov, V., Budzowska, M. & Walter, J. C. BRCA1 promotes unloading of the CMG helicase from a stalled DNA replication fork. *Mol. Cell* **56**, 174–185 (2014).
71. Langston, L. D. *et al.* CMG helicase and DNA polymerase epsilon form a functional 15-subunit holoenzyme for eukaryotic leading-strand DNA replication. *Proc. Natl Acad. Sci. USA* **111**, 15390–15395 (2014).
72. Kubota, T., Nishimura, K., Kanemaki, M. T. & Donaldson, A. D. The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Mol. Cell* **50**, 273–280 (2013).
73. Dungrawala, H. *et al.* The replication checkpoint prevents two types of fork collapse without regulating replisome stability. *Mol. Cell* **59**, 998–1010 (2015).
74. Garg, P., Stith, C. M., Sabouri, N., Johansson, E. & Burgers, P. M. Idling by DNA polymerase delta maintains a ligatable nick during lagging-strand DNA replication. *Genes Dev.* **18**, 2764–2773 (2004).
75. Merlet, J. *et al.* The CRL2LRR-1 ubiquitin ligase regulates cell cycle progression during *C. elegans* development. *Development* **137**, 3857–3866 (2010).
76. Zechiedrich, E. L., Khodursky, A. B. & Cozzarelli, N. R. Topoisomerase IV, not gyrase, decatenates products of site-specific recombination in *Escherichia coli*. *Genes Dev.* **11**, 2580–2592 (1997).
77. Baxter, J. *et al.* Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes. *Science* **331**, 1328–1332 (2011).
78. Bastia, D. & Zaman, S. Mechanism and physiological significance of programmed replication termination. *Semin. Cell Dev. Biol.* **30**, 165–173 (2014).
79. Dalgaard, J. Z. *et al.* Random and site-specific replication termination. *Methods Mol. Biol.* **521**, 35–53 (2009).
80. Hamperl, S. & Cimprich, K. A. Conflict resolution in the genome: how transcription and replication make it work. *Cell* **167**, 1455–1467 (2016).
81. Mohanty, B. K., Bairwa, N. K. & Bastia, D. The Tof1p–Csm3p protein complex counteracts the Rrm3p helicase to control replication termination of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **103**, 897–902 (2006).
82. Jaiswal, R. *et al.* Functional architecture of the Reb1–Ter complex of *Schizosaccharomyces pombe*. *Proc. Natl Acad. Sci. USA* **113**, E2267–E2276 (2016).
83. Ivessa, A. S., Zhou, J. Q. & Zakian, V. A. The *Saccharomyces* Pif1p DNA helicase and the highly related Rrm3p have opposite effects on replication fork progression in ribosomal DNA. *Cell* **100**, 479–489 (2000).
84. Martinez, P. & Blasco, M. A. Replicating through telomeres: a means to an end. *Trends Biochem. Sci.* **40**, 504–515 (2015).
85. Wu, P., Takai, H. & de Lange, T. Telomeric 3' overhangs derive from resection by Exo1 and Apollo and fill-in by POT1b-associated CST. *Cell* **150**, 39–52 (2012).
86. Chow, T. T., Zhao, Y., Mak, S. S., Shay, J. W. & Wright, W. E. Early and late steps in telomere overhang processing in normal human cells: the position of the final RNA primer drives telomere shortening. *Genes Dev.* **26**, 1167–1178 (2012).
87. Soudet, J., Jolivet, P. & Teixeira, M. T. Elucidation of the DNA end-replication problem in *Saccharomyces cerevisiae*. *Mol. Cell* **53**, 954–964 (2014).
88. Hogg, M. *et al.* Structural basis for processive DNA synthesis by yeast DNA polymerase varepsilon. *Nat. Struct. Mol. Biol.* **21**, 49–55 (2014).
89. Sfeir, A. J., Chai, W., Shay, J. W. & Wright, W. E. Telomere-end processing the terminal nucleotides of human chromosomes. *Mol. Cell* **18**, 131–138 (2005).
90. Yeeles, J. T., Poli, J., Marians, K. J. & Pasero, P. Rescuing stalled or damaged replication forks. *Cold Spring Harb. Perspect. Biol.* **5**, a012815 (2013).
91. Georgescu, R. *et al.* Structure of eukaryotic CMG helicase at a replication fork and implications to replisome architecture and origin initiation. *Proc. Natl Acad. Sci. USA* **114**, E697–E706 (2017).
92. Lujan, S. A., Williams, J. S. & Kunkel, T. A. DNA polymerases divide the labor of genome replication. *Trends Cell Biol.* **26**, 640–654 (2016).

Acknowledgements
The authors thank E. Low for critical feedback on the manuscript, and K. Labib and A. Gambus for sharing results before publication. J.C.W.'s work on termination is supported by US National Institutes of Health (NIH) grant GM80676. J.C.W. is an investigator of the Howard Hughes Medical Institute, USA.

Competing interests statement
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.