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Mechanisms of DNA replication termination

James M. Dewar¹ and Johannes C. Walter^{2,3,*}

¹Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37323, USA

²Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

³Department of Biological Chemistry, Howard Hughes Medical Institute, and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

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 finishes when converging replication forks meet. During this process, called 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 then briefly review the mechanism of termination in the bacterium *Escherichia coli* and in simian virus 40 (SV40) before focusing 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.

Introduction

Genomic DNA replication can be divided into three general phases: (1) Initiation, in which the origin of DNA replication is unwound by the replicative DNA helicase (Figure 1A-B). (2) Elongation, in which forks copy the chromosome using semi-conservative DNA synthesis (Figure 1C-D). (3) Termination, when converging replication forks meet (Figure 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 studied extensively^{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 just as abundant as initiations, occurring approximately 50,000 times during a typical S phase of mammalian cells⁵.

*Correspondence: johannes_walter@hms.harvard.edu.

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At least five processes are unique to the final phase of replication and thus can be considered part of replication termination. The first concerns the resolution of topological stress. Unwinding of the parental duplex leads to overwinding of the unreplicated DNA, leading to the formation of positive supercoils ahead of the fork (Figure 1C). If too many supercoils accumulate, further unwinding becomes energetically unfavorable and replication ceases. There are two ways to dissipate positive supercoils. The first involves relaxation of supercoils by type I or type II DNA topoisomerases⁶. 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} (Figure 1E), which can be resolved by Type II, but not 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 domain⁹. In this view, as replication progresses, the resolution of topological stress would become increasingly reliant on the formation and subsequent removal of pre-catenanes. Importantly, at some point, the parental DNA between converging forks becomes too short to supercoil (Figure 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 absolutely dependent on the formation of pre-catenanes (Figure 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 removal of pre-catenanes were disrupted, forks would stall at a very late stage of replication due to accumulation of topological stress.

The second process unique to replication termination is the meeting of converging replication forks, which we call “encounter” (Figure 1E). It is presently unclear whether this involves a steric clash between replisomes and whether such a clash impedes further steps in termination. Third, replisomes dissociate from the DNA in a process called “disassembly” (Figure 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 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 still engaged in replication. Key questions are thus whether the replisome is actively disassembled, when this happens and what is the effect of defective disassembly. Fourth, DNA synthesis is completed through gap filling (Figure 1E-F). At the moment of 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 in, and the last Okazaki fragment is processed. Presently, it is unclear whether gap filling requires replisome disassembly and whether maturation of the last Okazaki fragment occurs via the same mechanism as during replication elongation. Finally, copying the last

turn of parental duplex creates a new catenane and also converts any pre-catenanes to catenanes (Figure 1F). All of these catenanes have to be decatenated (resolved) prior to chromosome segregation (Figure 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 begin by summarizing 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, which include the first evidence of an active replisome unloading mechanism.

Termination in *Escherichia coli*

The circular *E. coli* chromosome, comprising 4.6 million basepairs of DNA, is replicated from a single origin of replication, *oriC* (Figure 2A)². Two forks are established, each containing a hexameric replicative helicase, DNA synthesis protein B (DnaB), which unwinds parental DNA by encircling it 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 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/min 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) and comprise potent and polar replication fork barriers (Figure 2A, reviewed in¹¹). The *ter* sites are oriented such that the leftward fork can pass the first five *ter* sites it encounters, which are marked as red arrowheads in Figure 2A, but stalls at the five blue sites. Conversely, the rightward fork passes through the *ter* sites marked as blue arrowheads but stalls at the red sites. In this way, forks can enter but not leave the termination zone.

The function of Tus-*ter* complexes in replication termination remains unclear. It has been debated¹¹ whether fork encounter occurs *after* one of the two forks has already stalled at a *ter* site (Figure 2Ba), or whether fork encounter occurs between two *ter* sites (Figure 2Bb). Some forks clearly collide with the non-permissive face of a Tus-*ter* complex, as judged by 2-D gel electrophoresis¹². However, when the *tus* gene is deleted (*tus*⁻), the location of most fork fusions remains ~10,000 bp to the right of *terC*^{13,14}. Therefore, most forks appear to converge between *ter* sites C and A (Figure 2Bb). Surprisingly, *tus* strains have no abnormal growth phenotypes¹⁵, suggesting that the Tus-*ter* system is not an integral part of the termination machinery, but instead has other roles (see below).

How do the five steps of replication termination outlined in Figure 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 decatenation of the fully replicated daughter molecules¹⁶ (Figure 2Ca,Cb). Evidence also indicates that TopoIV is required to resolve pre-catenanes and thereby allow 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 judged by deep sequencing of genomic DNA¹⁴. 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 (Figure 2Cb). The flap is normally degraded or remodeled and the gap is subsequently filled in (Figure 2Cc). Pol I may use its 5' to 3' exonuclease activity to remove the RNA primer of the last Okazaki fragment²¹, as seen during replication elongation, to facilitate ligation (Figure 2Cc). If the flap is not removed, two replication forks are established following strand invasion and priA-dependent loading of DnaB (Figure 2D). Notably, the extent of re-replication is greatly increased in the absence of Tus^{14,19,21}. Therefore, it appears 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 opposing fork's leading strand, it unwinds this strand (Figure 2Cb). However, biochemical studies indicate that in this situation, DnaB would pass over the 3' end and keep translocating along dsDNA without further DNA unwinding²². 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 leading and lagging strand polymerases are physically coupled to DnaB, cause a steric clash that requires replisome disassembly before gaps can be filled? The model 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 (Figure 2Cb). Further work is needed to address these issues, including why *E.coli* termination appears to be so susceptible to re-initiation.

Termination in simian virus 40

Replication termination has been studied extensively in the context of the mammalian DNA tumor virus SV40, whose small circular (plasmid) chromosome comprises 5,200 bp (Figure 3A). SV40 encodes its own replicative helicase, large T antigen (T-ag), which cooperates with mammalian host replication factors to replicate the SV40 chromosome²³. Two replication forks are established at the origin and terminate on the opposite side of the plasmid (Figure 3A). Relocation of the origin leads to a corresponding shift in the termination zone²⁴, indicating that SV40 lacks genetically encoded termination sites.

Two long-lived intermediates have been detected during SV40 replication termination. The first is a 'late theta' structure^{25–28}, in which all but the final ~450 bp of the SV40 chromosome is replicated (Figure 3Ba)²⁹. 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 (Figure 3Bb). In

cell-free extracts, replicated SV40 plasmid dimers contain 5–20 catenanes (Figure 3Bc)^{30,31}, consistent with the formation of precatenanes during fork convergence (Figure 3Bb). Catenated plasmid dimers are ultimately resolved into circular monomers (Figure 3Bd). The final stage of SV40 DNA synthesis requires topoisomerase II (Topo II)^{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 (Figure 3Cc)³⁴, but what causes this gap is a matter of speculation (see below). Importantly, decatenation (Figure 3Cc) and gap filling (Figure 3Cd) appear to be mechanistically independent events³⁰.

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 convergence³⁵. However, such a model does not explain how the last 20% of parental DNA is unwound, and it is incompatible with evidence that complete SV40 DNA replication can be reconstituted in a defined system where T-ag is the only DNA helicase³⁶. An early model of DNA unwinding by T-ag proposed that it encircles the DNA duplex and translocates along it³⁷. In this view, T-ag molecules would stall at encounter and would have to be disassembled prior to gap filling. However, more recent work indicates that T-ag, like other replicative DNA helicases, translocates along one strand of the DNA³⁸ (Figure 3Ca), suggesting that converging T-ag molecules should be able to pass each other (Figure 3Cb,c). T-ag stalling at the downstream Okazaki fragment might inhibit ligation and explain the persistence of the 60 nt ssDNA after decatenation³⁴. To understand SV40 termination more fully, it will be crucial to determine when exactly T-ag dissociates from DNA, whether this is an active process, and what are the 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 they are linked to replisome disassembly.

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 to the active CMG helicase, which is composed of the cell division cycle 45 (CDC45), MCM2–7 and the four-subunit complex Go-Ichi-Ni-San (GINS). CMG unwinds the origin, 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, but termination is inefficient⁴³. 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 events that underlie eukaryotic replication termination, including the active disassembly of the replisome.

Genomic distribution of termination sites—We first 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 elements⁴⁶. More recently, Okazaki fragments were mapped genome-wide in unsynchronized budding yeast cells to identify fork merger zones⁴⁷. This analysis showed that termination events generally occur midway between origins and that the more active two origins are, the better defined the termination zone between them. Alteration of origin firing (activation) caused predictable changes in termination zones, consistent with the large majority of termination events being sequence non-specific and largely dictated by initiation patterns^{47,48}. Similar conclusions were reached from Okazaki fragment mapping in mammalian cells⁴⁹. The absence of specific termination sites is well suited to deal with the significant 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 down during fork convergence, and studies in both SV40 and *E. coli* suggested that fork convergence requires the activity of Topo II. To study eukaryotic replication termination in frog egg extracts, forks were stalled at the outer edges of a ~500 bp-long array of LacI molecules, followed by IPTG addition to induce locus-specific and synchronous termination events⁴⁴. No slowing in the rate of DNA synthesis was observed during duplication of the final 500 bp of DNA. Although some topological stress may have dissipated prior to disruption of the LacI barrier, completion of replication still required the removal of ~50 supercoils. These results suggest that in this cell-free system, the resolution of topological stress is not rate-limiting for fork convergence, presumably owing to the efficient formation of pre-catenanes (Figure 4A). Accordingly, DNA catenation was detected immediately following fork convergence⁴⁴, as previously reported^{50,51}. Replicon size does not affect the number of catenanes formed⁵², suggesting precatenanes are primarily formed during replication termination rather than elongation^{9,53}. Strikingly, unlike what is observed in *E. coli* and SV40, fork convergence and synthesis completion do not require Topo II in egg extracts or yeast^{44,54,55}. In the absence of Topo II, fully replicated daughter plasmids are generated, and they are highly catenated, indicating that resolution of precatenanes 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 and prokaryotic systems.

Replisome encounter—A critical question is what happens when converging CMG complexes meet. In frog egg extracts, nascent leading strands pass each other without detectable pausing, followed by rapid ligation of all nascent strands (Figure 4), indicating that during fork encounter, there is no steric clash or if there is, it is very short-lived. This is consistent with our current understanding of replisome architecture. First, replisome-associated CMG appears to interact primarily with the leading strand template⁵⁶. Therefore, the converging CMG complexes will encounter each other on different strands (Figure 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 (CMG, Pol ϵ , etc.) and the lagging strand replication machinery (Pol δ , proliferating cell nuclear antigen (PCNA), flap endonuclease 1 [FEN1], DNA ligase, etc.),

although Pol α -primase (Pol α) does bind weakly to a CMG complex in yeast⁵⁷. Such a separation between leading and 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 would likely be deleterious for genome stability. Curiously, when CMG encounters a covalent DNA-protein (~40 kDa) complex on the lagging strand template, it stalls for a few minutes⁵⁸. This observation raises the interesting possibility of specific evolutionary adaptations that prevent a clash during 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^{45,59} (Figure 4E). In budding yeast, MCM7 polyubiquitylation and CMG dissociation both require the E3 ubiquitin ligase SCF^{Dia2} (Skp, Cullin, E-box containing complex associated with Digs Into Agar 2 [Dia2])⁵⁹, strongly suggesting that MCM7 ubiquitylation is causally linked to CMG unloading. Recently, CRL2^{Lrr1} (Cullin RING Ligase 2 associated with Leucine Rich Repeats 1 [Lrr1]) was identified as the ubiquitin ligase that promotes MCM7 ubiquitylation and CMG unloading at the end of S phase in worms and frogs^{60,61}. SCF^{Dia2} may be constitutively associated with replisomes in yeast⁶², whereas frog CRL2^{Lrr1} only binds to terminating replisomes⁶⁰. Once CMG is ubiquitylated, it is removed from chromatin by the ATPase p97 (also known as VCP)^{45,59,63} (Figure 4E,F), which cooperates with diverse co-factors to extract ubiquitylated proteins from their local environments⁶⁴. Data from frogs indicate that ubiquitylated MCM7 is not degraded by the proteasome after chromatin extraction, suggesting that ubiquitylated CMG is recycled after disassembly⁶⁵. Yeast cells lacking Dia2 exhibit constitutive activation of the replication checkpoint, sensitivity to DNA damaging agents and gross chromosomal rearrangements^{66–68}. In the absence of CRL2^{Lrr1}, worm CMG persists on chromatin until prophase, when it is unloaded by a second pathway involving p97 and UBX domain containing 3 (UBXN-3, the worm ortholog of human FAS-associated factor 1, FAF1)⁶¹. Importantly, combined knock-down of LRR-1 and UBXN-3 stabilizes CMG on chromatin until metaphase and is synthetically lethal, suggesting replisome unloading is essential for viability⁶¹. 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 whether these 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 in and ligated (Figure 4D-F)⁴⁴. As such, CMG unloading is one of the latest known events in eukaryotic replication termination, occurring once CMG is associated with dsDNA. Most likely, converging CMGs pass each other and keep translocating along the leading strand template until they reach the downstream Okazaki fragment, whereupon they pass onto dsDNA (Figure 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 5' flap, as reported also for DnaB^{69,70}. 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, the data suggest that MCM7

ubiquitylation occurs when CMG encircles double-stranded DNA. Importantly, synthesis completion is unaffected when CMG unloading is inhibited, consistent with unloading being a later event in termination^{44,60}.

A critical unresolved issue concerns the trigger for CMG unloading. Given that MCM2–7 loading (and therefore CMG assembly) in S phase is prohibited to prevent re-replication¹, premature CMG removal from active forks must be prevented to avoid fork stalling and breakage. An attractive model is that the presence of dsDNA in the central channel of CMG leads to recruitment of CRL2^{Lrr1} (or in yeast activation of already bound SCF^{Dia2}) owing to a conformational change of CMG. Remarkably, induction of SCF^{Dia2} expression in G1 can unload CMG complexes that remained on chromatin from the previous S phase⁵⁹, 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 ssDNA⁵⁶ (Box 1). To discriminate between CMG complexes during termination and licensed MCM2–7 complexes, which also encircle dsDNA, we speculate that the ligase might detect the presence of CDC45 and/or GINS, or 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 inter-strand crosslink (ICL)^{56,71}. Although this pathway involves MCM7 ubiquitylation and p97 (REF.64)⁶⁵, it operates on CMG complexes that encircle single-stranded DNA on either side of an ICL, and unlike termination-dependent CMG unloading, it requires BRCA1 bound to BRCA1-associated RING domain protein 1 (BARD1) (REFS 64, 70)^{65,71}. Therefore, the mechanisms of CMG unloading during termination and ICL repair are clearly distinct.

How does the rest 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 ^{39,62,72}. It is therefore likely that numerous replication proteins are unloaded passively, as an indirect consequence of CMG unloading. Consistent with this idea, CMG and Pol ϵ dissociate with similar kinetics⁴⁴, and blocking CMG unloading leads to the retention of most RPC components at the fork, including Pol ϵ ⁶⁰. To determine whether SCF^{Dia2} or CRL2^{LRR1} target other replisome components, it will be important to test whether 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 ELG1 (also known as ATAD5)^{73,74}, 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, whereupon the two are rapidly ligated⁴⁴ (in contrast to the situation in SV40 DNA, where gaps persist). This observation implies that neither the resolution of topological stress nor CMG unloading are 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 complex⁷², away from the junction⁶⁰. This is potentially advantageous, because unlike Pol δ , Pol ϵ does not functionally cooperate with FEN1 for Okazaki fragment processing⁷⁵. Thus, removal of Pol ϵ should make room for Pol δ and facilitate processing. This mechanism has a potential disadvantage. If CMG is able to translocate a significant distance along dsDNA before being unloaded, it would promote re-replication if it encounters a downstream Okazaki fragment with a 5' flap, analogous to the situation in *E. coli* (Figure 2Da). In possible agreement with this possibility, loss of CRL2^{Lrr1} in worms appears to induce re-replication⁷⁶. In the future, it will be crucial to assess whether short stretches of re-replication normally occur in healthy cells or in cells where a component of the Okazaki maturation machinery, such as FEN1, has been compromised.

Decatenation of replicated chromosomes—When the last turn of parental DNA duplex is unwound, the daughter molecules end up catenated via one interwinding (Figure 1F). In addition, any pre-catenanes are automatically converted into catenanes⁹. The resolution of catenanes is unlikely to be mechanistically distinct from the unlinking of precatenanes behind replication forks¹⁸. We note that both *E. coli* and yeast are capable of carrying out a mechanistically distinct decatenation process, which is driven by supercoiling of the chromosome^{77,78}. The mechanism described in yeast requires Condensin loading and spindle formation⁷⁸, which only occur during mitosis. Therefore, it is unlikely to be extensively employed during replication termination, which occurs during S phase.

Site-specific replication termination—Although the vast majority of eukaryotic termination events appear to be sequence non-specific (see above), at least two classes of site-specific termination exist^{79,80}. 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). Like 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 transcription machineries, which have the potential to cause genome instability⁸¹. Although the mechanism of fork arrest at the rDNA RFB is quite well defined^{82,83}, 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 replication⁴⁴. Therefore it is likely that the replisome from the fork stalled at the rDNA RFB can restart once the converging fork displaces the terminator protein. Most likely, replisomes then pass each other and terminate replication using the same mechanism employed at other loci (Figure 4b-f). In budding yeast, the helicase rDNA recombination mutation protein 3 (Rrm3) is required for progression of replication forks past protein-DNA complexes. Interestingly, although *rrm3* strains exhibit a ~2-fold increase in replication fork stalling at Fob1-*Ter*, they show a ~10-fold accumulation of converged forks at this RFB, suggesting a specific role for Rrm3 in fork convergence⁸⁴. Importantly, *rrm3* strains exhibit no genome-wide defects in replisome disassembly⁵⁹, suggesting a specialized role

for Rrm3 at the rDNA RFB, perhaps in displacing Fob1⁸². 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 aging are not discussed as they have been reviewed elsewhere^{79,80}.

The second class of site-specific termination events occurs at telomeres⁸⁵. Here, replication ceases when the fork reaches the end of the chromosome. Importantly, when the fork copies the telomere, the leading strand appears to be extended to within a few nucleotides of the chromosome end^{86–88}, similar to the run-off DNA synthesis exhibited by purified Pol ϵ ⁸⁹. Together, these observations suggest that CMG slides off the end of the leading strand template, allowing Pol ϵ to reach the end of the chromosome⁹⁰. Given the evidence that Pol α is tethered to the replisome through CMG^{57,60}, Pol α should dissociate with CMG, thereby preventing new priming. This might explain why the amount of DNA lost on the lagging strand in humans is roughly equivalent to the size of an Okazaki fragment⁸⁷. The above model predicts that replisome dissociation at telomeres does not require MCM7 ubiquitylation, and it will be important to test this 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 determine the similarities and differences between them. Based on 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 are 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 end game of DNA replication might be as highly regulated as initiation, albeit by ubiquitylation rather than by the phospho-regulatory mechanisms that are employed during replication initiation. Similarly, do 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 would likely also occur during replication elongation, which might explain the existence of helicase reloading pathways in bacteria but not eukaryotes⁹¹. 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 they stall upon contact, is that because of the physical coupling between the leading and 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.

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Glossary

Catenanes

double-stranded intertwinings between two DNA molecules

Fork stalling

a pathological situation where replication fork progression is impaired

Okazaki fragment

a short DNA fragment synthesized on the lagging strand template

Origin of DNA replication

the location at which replicative helicases are loaded onto DNA, which are generally site-specific in bacteria and yeast, but not in metazoa

Pre-catenanes

catenanes that are located behind the replication fork, on recently-replicated DNA

Replication forks

spread DNA structures where the replisome is engaged in DNA synthesis

Replicon

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

Replisomes

the collections of proteins involved in DNA replication at the replication fork

Replisome progression complex

a large assembly of proteins bound directly or indirectly to the replicative CMG helicase

Supercoil

a superhelical twist of the DNA duplex that arises 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

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

Biographies:

Johannes Walter obtained his Ph.D. from Yale University and was a post-doctoral fellow at UCSD, where he developed the first soluble system that supports vertebrate DNA replication. He is now a Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and an investigator of the Howard Hughes Medical School. His laboratory uses frog egg extracts to elucidate mechanisms of DNA replication and replication-coupled DNA repair.

James Dewar performed his graduate studies on telomere metabolism at Newcastle University, England. He was a postdoctoral fellow in Johannes Walter's lab at Harvard Medical school, USA, where he developed a reversible replication barrier approach to study DNA replication and repair. He is now an Assistant Professor in the Department of Biochemistry at Vanderbilt University. His lab uses frog egg extracts to investigate DNA replication termination and telomere replication.

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Online summary

- Termination of DNA replication occurs when two replication forks meet on the same stretch of DNA, during which the following events occur, though not necessarily in this order: forks converge until all intervening DNA is unwound; any remaining gaps are filled in and ligated; catenanes are removed; replication proteins are unloaded.
- In eukaryotes, most termination sites are determined stochastically by the location of replication initiation sites. In prokaryotes, termination generally occurs at a specific locus.
- Replication termination can be a problematic process. Termination of simian virus 40 (SV40) replication involves stalling of converging forks and in bacterial termination is prone to inducing re-replication. By contrast, fork stalling or re-replication have not been observed during unperturbed termination in eukaryotes.
- Topological stress accumulates between converging forks and is relieved by generation 'pre-catenanes', which are removed by Type II topoisomerases. During bacterial and SV40 termination, Type II topoisomerases are required for fork convergence, but in eukaryotes they are dispensable for this purpose.
- After forks converge, any remaining catenanes are removed by a Type II topoisomerase. In eukaryotes, gaps are readily filled in by extension of the leading strands, but in bacteria and SV40 this process is less defined.
- In eukaryotes, a dedicated replisome removal pathway was recently identified, which operates late during termination, after the DNA is fully replicated. It is unclear whether any comparable pathway exists in prokaryotes.

Figure 1: Steps in DNA replication

Generic illustration of replication initiation (A-B), elongation (C-D), and five events that are unique to replication termination (D-G). The replicative DNA helicase is depicted without reference to a specific translocation mechanism; RNA primers are in red. The order of the termination events is hypothetical.

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 shown as red and blue arrowheads. The termination zone is underlined in red. In the box, the green arrow shows a replication fork passing through a *ter* site in the permissive orientation, and the red arrow shows a fork stalling at a *ter* site in the non-permissive orientation. (B) Two scenarios of fork stalling in the termination zone. (a) The rightward fork (Fork 1) arrives first and stalls at *terC*, followed by the arrival of the leftward (Fork 2). (b) The two forks arrive to the termination zone contemporaneously and meet

between *terC* and *terA*. **(C)** Possible mechanism of *E. coli* replication termination. (a) Forks converge between *ter* sites with the formation of pre-catenanes. (b) 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, gaps are filled in and the final Okazaki fragment is processed by DNA polymerase I (Pol I). (c) 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. (a) If the 3' flaps are not removed or remodeled, a new replication fork is established, which prevents the completion of termination. (b) The free end re-invades the sister chromatid using recombination protein A (RecA) and RecBCD, which establish a new replication fork. (c) The Holliday junction is resolved and DnaB is re-loaded onto the fork by primosomal proteins A (PriA) and PriB.

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 termination zone (underlined in red). (B) Late stages of SV40 DNA replication. (a) Late theta intermediate, when fork convergence begins. (b) Fork convergence, when superhelical stress is dissipated by the formation of pre-catenanes. (c) Catenated dimers are generated when pre-catenanes are converted to catenanes at the end of replication. (d) Decatenation produces two circular monomers. (C) Hypothetical mechanism of SV40 replication termination. (a) When forks come within 450 bp of each other, they

stall, possibly owing to reduced formation of pre-catenanes. (b) 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. (c) The helicases pass each other and stall at the downstream Okazaki fragment, accounting for the single-stranded DNA gap that persists on replicated molecules. (d) T-ag is unloaded, the remaining gaps are filled in. (e) The catenanes are removed, yielding fully replicated and decatenated daughter chromosomes.

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Figure 4: Model of eukaryotic replication termination

(A) After copying most of the replicon, forks come too close to each other to allow 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 precatenanes. An end-on view of CMG illustrates the presence of single-stranded DNA in its central channel. (B) The encounter causes no detectable fork stalling, implying that converging CMGs bypass each other. After bypass, CMG helicases keep translocating until they reach a downstream Okazaki fragment. (C) The CMG helicases

pass over the ssDNA–dsDNA junction and keep moving on dsDNA (see end-on view). **(D)** The leading strand is extended to the downstream Okazaki fragment. The last 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} or CRL2^{Lrr1}. The ubiquitylated MCM7 is extracted from chromatin by the ATPase p97 ATPase. **(F)** Catenanes are removed. CMG, CDC45–MCM–GINS

Box 1: Eukaryotic replication initiation and elongation: the basics

Here we provide a brief summary of eukaryotic DNA replication initiation and elongation (reviewed in ^{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 heterohexameric composed of the related ATPases MCM2–MCM7 and serves as the motor of the replicative helicase. Within pre-RCs, two inactive MCM2–7 complexes encircle double-stranded DNA, with their N-terminal tiers oriented towards each other to form a tight dimer interface. In S phase, a subset of pre-RCs undergoes activation by cyclin-dependent kinase (CDK), Dbf4-dependent kinase (DDK) and many accessory factors, leading to the binding of two helicase co-factors, 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; the trailing edge of CMG is formed by the C-terminal lobe of MCM2–7⁹². 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 phase¹.

In yeasts, although origin sequences are well-defined, initiation is partly stochastic, so that the program 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 programs that are even more stochastic than in yeasts. The replisome is a macromolecular assembly 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 synthesized by Pol δ ⁹³ (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 a ~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' of another, Pol δ performs strand displacement synthesis (Figure 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 remodeling factors, checkpoint signaling 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 appear not to form a stable complex in eukaryotes.