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Termination of DNA Replication in Prokaryotes

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Abstract

Most bacteria and archaea have circular chromosomes, in which DNA replication begins at a site known as an origin of replication. Double-stranded DNA unwound at the origin creates two replication forks that are engaged by DNA polymerase complexes (replisomes) that advance each fork and proceed in opposite directions away from the origin, copying the original strands. Termination of DNA replication occurs when the two forks meet and fuse, creating two separate double-stranded DNA molecules. In the well-studied bacteria *Escherichia coli* and *Bacillus subtilis*, this occurs in the terminus region, which is situated diametrically opposite the origin. Failure to terminate chromosome replication correctly can lead to problems with genome function and stability, including DNA over-replication. In contrast, some archaea have multi-origin chromosomes and do not appear to specifically regulate the location of termination.

Keywords:

- DNA replication;
- replication termination;
- replication fork arrest;
- DNA terminators;
- replication terminator protein;
- termination utilization substance;
- catenanes

Key Concepts:

- Termination of DNA replication occurs when two oppositely-orientated replication forks meet and fuse, to create two separate and complete double-stranded DNA molecules.
- In circular bacterial chromosomes, termination is restricted to a region called the terminus region, located approximately opposite the origin of replication.
- A replication fork trap is an opposing arrangement of unidirectional replication terminator (*Ter*) sites in a region of DNA, which allows replication forks to enter the trap from either direction, but not exit it.
- Failure to terminate bacterial chromosome replication correctly results in chromosome over-replication and genome instability.

Introduction

Chromosome replication and the cell cycle of bacteria such as *Escherichia coli* and *Bacillus subtilis* shows significant differences to that of eukaryotic cells. Whereas eukaryotes typically have multiple linear chromosomes with cell cycle events are temporally distinct and under the control of strict checkpoint systems, these bacteria have a single circular chromosome (~4-5 Mbp) and exhibit overlapping cell cycle events. The DNA, which forms a compact so-called nucleoid inside the cell, is duplicated by two replication machineries, or replisomes, that start from a unique site called the origin of chromosome replication (*oriC*), and then move in opposite directions around the circular chromosome, processively replicating the DNA to create two identical chromosomes (Beattie and Reyes-Lamothe, 2015; Jameson and Wilkinson, 2017). The replicating DNA is continuously segregated towards opposite cell halves, while replication of the remainder of the chromosome is still ongoing (Reyes-Lamothe et al., 2012). Remarkably, many bacteria can also simultaneously carry out multiple rounds of chromosome replication, in order to replicate DNA fast enough during high growth rates, *i.e.* a new round of chromosome replication is started while previous rounds of duplication and segregation are still ongoing. In nutrient-rich conditions, this allows a single *E. coli* cell to have up to 3 division events per hour, even though it takes at least 40 minutes to complete replication of the chromosome (Helmstetter et al., 1968; Zaritsky and Woldringh, 2015). A pair replication forks originating together

from *oriC* eventually approach one another and fuse within a region diametrically opposite *oriC*, called the terminus region. After termination, the two daughter chromosomes are further resolved through post-replicative processing if they remained interlinked or joined together as a chromosome dimer (Lesterlin et al., 2004; Reyes-Lamothe et al., 2012). These processes are temporally and spatially coordinated with the formation of a cell division septum at mid-cell between the two daughter chromosomes (Reyes-Lamothe et al., 2012; Zaritsky and Woldringh, 2015).

The emphasis in this review will be on the approach and fusion of the forks in the terminus region, the importance of its regulation, and how termination relates to post-replicative chromosome resolution.

The bacterial chromosome terminus region and replication fork trap

In *E. coli* and *B. subtilis*, the location of fork fusion is restricted to the centre of the terminus region by several replication pause sites (explained further below). In contrast, in diverse eukaryotes and archaea that have multiple replication origins per chromosome, fork fusion does not appear to be controlled by specific pause sites in many cases and instead occurs at random sites within fork fusion zones between origins (Duggin et al., 2011; Gambus, 2017; Hawkins et al., 2013; Samson et al., 2013).

The terminus region in both *E. coli* and *B. subtilis* functions as a "replication fork trap". This is due to the presence of a series of short DNA sequences, each having the potential to pause replication fork progression. Each of these so-called DNA terminator (or *Ter*) sites is asymmetric; it can arrest a fork approaching from one direction but not the other. The positioning and orientation of the terminators creates two opposed groups that would allow forks to enter but not exit the terminus regions, as shown in Figure 1. In *E. coli* the identified terminators are distributed over 42.5% of the chromosome, whereas in *B. subtilis* the spread is much narrower (9.9%). However, in *E. coli* only the inner-most 4 sites are substantially involved in DNA replication fork arrest during normal chromosome replication (Duggin and Bell, 2009), and these sites are therefore considered to constitute the primary fork trap (spanning 8.7% of the chromosome). Nevertheless, the two opposed subgroups of terminators flank the central portion of the terminus region, such that those in one subgroup (*TerC*, *B*, *F*, *G* and *J* in *E. coli*) can arrest a clockwise-moving fork, and those in the other subgroup (*TerA*, *D*, *E*, *I* and *H* in *E. coli*) can block a anticlockwise-moving fork. How the fork trap operates can be understood by considering, for example, the clockwise fork from *oriC* in *E. coli*; after initially passing through the permissive orientations of *TerH, I*, *E*, *D* and *A*, it will encounter blockage at *TerC* and be arrested for a finite period (a few minutes or so). If the anticlockwise fork does not progress to meet the clockwise fork while the latter is stalled, and thus achieve termination of replication, the clockwise fork proceeds to meet *TerB*, where it stops again, and so on until fusion with the anticlockwise fork is finally achieved. A similar situation would arise if the anticlockwise fork were to arrive first (initially encountering *TerA*). Thus,

the multiple terminators appear to function as backups to ensure that any fork entering the terminus region does not exit it. Because of the positioning of *TerC* relative to the origin of replication and the other terminators, it is generally the first to be encountered by a replication fork (the clockwise one) and is considered to be the most frequently used terminator site in *E. coli* (Duggin and Bell, 2009). Of the nine *Ter* sites in *B. subtilis* (Figure 1b), *TerI* at 172° is generally the first to be encountered by a fork (the clockwise one) and is probably the most frequently used in this organism. In both *B subtilis* and *E. coli*, the outer terminators are probably used only rarely (Duggin and Bell, 2009; Griffiths and Wake, 2000).

<FIGURE 1 NEAR HERE>

DNA Terminator (*Ter*) sites

In order to arrest a replication fork, a DNA terminator must bind a specific terminator protein, Tus (terminus utilization substance) in *E. coli*, and RTP (replication terminator protein) in *B. subtilis*. The genes encoding these proteins (*tus* and *rtp*) are themselves located within the terminus region (Figure 1). Their expression is auto-regulated through binding of the terminator protein to upstream terminators (*TerB* in *E. coli* and both *TerI* and *TerII* in *B. subtilis*) located very close to the gene's promoter, negatively influencing transcription; this keeps the amount of the terminator proteins low in each cell (in the order of 100 copies per cell) (Ahn et al., 1993; Natarajan et al., 1991; Roecklein et al., 1991; Roecklein and Kuempel, 1992).

The *Ter* sites in *E. coli* (Figure 1a) were defined as a 23 bp long sequence, but show strongest conservation and importance for Tus binding between residues 6 and 19 inclusive (Figure 1b) (Coskun-Ari and Hill, 1997). A single molecule (monomer) of Tus binds specifically to each sequence (Figure 2a). The *B. subtilis Ter* sequences are completely different to those in *E. coli*. *B. subtilis Ter* sites show conservation over a 28 bp long sequence (Figure 1d), and contain two overlapping binding sites—an 'A site' and a 'B site'—each of which is capable of binding a dimer of RTP (Figure 2b). Both the A and B sites must bind a dimer of RTP in order to confer fork arrest activity. However, only replication forks approaching the B-site end of the *Ter* sequence are arrested, accounting for the polarity of action (Smith and Wake, 1992). Interestingly, both naturally occurring and synthetic terminators exist on *B. subtilis* plasmids that are composed of two B sites instead of an A and a B site; these terminators have the ability to arrest DNA replication forks approaching from either direction (Meijer et al., 1996; Smith et al., 1996). An equivalent 'bidirectional' terminator has not been discovered in *E. coli*. The lack of sequence or structural similarity between the terminator proteins of *E. coli* and *B. subtilis* and the way in which they recognize their cognate DNA binding sites suggests the two systems have evolved independently of one another (Wake and King, 1997). While closely related species and plasmids harboured by these two organisms also contain homologous replication pause sites in each case

(Griffiths et al., 1998; Hidaka et al., 1988; Meijer et al., 1996; Roecklein et al., 1991), their lack of sequence conservation across diverse bacteria means that it is still unknown how universal the existence of such terminus-region pause sites is across the bacterial domain. However, their apparent independent evolution in the Gram-positive *B. subtilis* and Gram-negative *E. coli* suggests that fork traps or pause sites have significant roles in regulating circular chromosome replication and may therefore be expected to be seen in other bacteria.

Mechanisms of DNA replication fork arrest

Tus: the replication terminator protein of *E. coli*

Stopping a highly complex replisome that moves with speeds between 650 and 1000 bp s^{-1} is no mean feat. In both yeast and human cells Tus-*Ter* complexes were successfully used to block DNA replication (Larsen et al., 2014; Willis et al., 2014). Tus is a monomeric protein of 36 kDa binds that very tightly to its cognate terminators; the Tus-*TerB* dissociation constant (K_D) was measured at 3.4×10^{-13} mol.L^{-1} and the complex ha a half-life of 550 min under the conditions studied (Hill et al., 1989). Despite the very tight binding, a single Tus-*Ter* complex is not sufficient to cause significant arrest DNA replication in eukaryotic cells, and between 3 and 7 tandem copies of *Ter* were used to observe arrest (Larsen et al., 2014; Willis et al., 2014). Furthermore, the *E. coli* Tus-*Ter* complex operating in *B. subtilis* is only 1-2% efficient in fork arrest compared to arrest of its native *E. coli* replisome (Andersen et al., 2000). Although it is possible that the binding affinity of Tus-*Ter* is weaker in the non-native cell environments, these observations may indicate that specificity features other than the Tus-*Ter* binding affinity could contribute towards stopping a replisome. Also, high-affinity binding of Tus-*Ter* alone cannot explain the polar (unidirectional) fork-arrest activity observed.

The three-dimensional structure of a Tus-*Ter* complex, determined using X-ray crystallography (Kamada et al., 1996a), provided clues towards understanding how the complex elicits polar fork-arrest activity. Tus is organized into a large N-terminal domain and a smaller C-terminal domain (Figure 2). Two very long β strands join these domains, and are responsible for recognition and tight binding of the *Ter* sequence, through extensive DNA major-groove contacts. Tus holds the DNA in a girth-like manner so that the DNA is flanked and somewhat enveloped by the two large domains. It is clear from the crystal structure that the replisome would encounter a very different surface of the Tus-*Ter* complex depending on the direction in which it was travelling—but how does this cause polar fork arrest? It was suggested that the bulky α-helical domains at the blocking end of the complex could sterically protect the specific Tus-*Ter* interactions that are clustered towards the permissive end, thereby sterically preventing the replisome from disrupting the Tus-*Ter* complex and passing through. From the other direction, the asymmetric protein-DNA contacts are exposed to disruption by the unwinding activity of the ring-shaped DnaB helicase encircling the lagging strand template the at the forefront of the

replisome. This model thus suggested that Tus-*Ter* acts as a directional 'molecular clamp' (Kamada et al., 1996a; Wake, 1996). This relatively non-specific mechanism could account for the observations that very different DNA unwinding and sliding proteins (other than the replisome) are arrested in a polar manner by Tus-*Ter in vitro*, depending on the strand and directionality of protein translocation (Bedrosian and Bastia, 1991; Hidaka et al., 1992; Lee and Kornberg, 1992; Lee et al., 1989; Pandey et al., 2015).

Are there any other mechanisms that contribute to fork arrest and help to arrest the full *E. coli* replisome *in vivo*, accounting for the functional specificity noted above? It has been proposed that specific binding between the blocking side of Tus-*Ter* and the DnaB helicase could inhibit DNA unwinding and underpin the mechanism of fork arrest (Bastia et al., 2008; Mulugu et al., 2001). Point mutations of Tus have been discovered that reduce the effectiveness of fork arrest by Tus but leave its DNA-binding affinity apparently unaffected (Henderson et al., 2001). The existence of such mutations supports the idea that additional important interactions occur when the replisome approaches Tus-*Ter*. Interestingly, however, these interactions were found to occur between the forked DNA and Tus, rather than between the replisome proteins and Tus, as explained below.

<FIGURE 2 NEAR HERE>

As the replisome approaches Tus-*Ter* and unwinds DNA up to the blocking end, it was discovered that a conserved cytosine residue (GC(6)) near the blocking face, upon becoming single stranded, swivels and binds a cytosine-binding pocket in Tus and greatly stabilizes the resulting Tus-*Ter* forked complex (Figure 2a) (Mulcair et al., 2006). This locking effect was termed the "mousetrap" mechanism. In contrast, strand separation if forks are approaching the permissive face resulted in Tus-*Ter* destabilization (Berghuis et al., 2015; Mulcair et al., 2006). A further study identified that mutations to the GC(6) residue of *TerB* affected the duration and efficiency of the fork arrest on the T7 helicase-polymerase complex (Pandey et al., 2015). Furthermore, the replisomes that stopped/paused were those that were travelling more slowly, whilst the faster ones were more likely to progress through the Tus/*Ter* site, since fast replisomes can move through to the C6 base before C6 locks onto Tus, thus increasing the probability of replisome displacing Tus and passing through (Elshenawy et al., 2015; Pandey et al., 2015). Consistent with this, a DNA substrate with mismatched base-pairing around the cytosine region that should favour the formation of the lock, blocked more efficiently and could block the faster travelling replisomes as well (Elshenawy et al., 2015; Pandey et al., 2015) .

RTP: the replication terminator protein of *B. subtilis*

RTP is a winged-helix DNA-binding protein that form a dimer in solution at concentrations above ~10^{-6} M, and shows no sequence or structural similarity to Tus (Figure 2) (Bussiere et al., 1995; Kralicek et al., 1993; Pai et al., 1996). Each monomer contains four α helices and three β strands, with dimerization

occurring via an antiparallel coiled-coil interaction, giving RTP its symmetrical dimer quaternary structure. Four RTP monomers bind to each *Ter* sequence to generated the functional complex, which is considered to have two half-sites (one for each of two dimers) designated the A site and the B site (Lewis et al., 1990). The A site binds an RTP dimer much more weakly than the B site, and it is the B site end of the complex from which the replisome can be arrested (Smith and Wake, 1992). The RTP-bound B site alone cannot cause arrest, and it has been demonstrated that the highly cooperative binding by RTP, which greatly enhances the affinity of each RTP dimer in the complex, is necessary for fork arrest activity when forks approach the B site (Smith et al., 1996). Interestingly, RTP mostly contacts one the two strands over the A site in *Ter* DNA (Langley et al., 1993), *i.e.* the leading strand as a replication fork approaches. The helicase is expected to encircle and translocate along the lagging strand, suggesting that the strand asymmetry might be important in the ability of the replisome to disrupt the complex to proceed when approaching the A-site end, analogous to the mechanism suggested for asymmetric DNA strand contacts by Tus.

The structures of the RTP dimer in complex with B-site DNA sequences revealed the mode of DNA binding (Vivian et al., 2007; Wilce et al., 2001). The primary sequence-specific contacts are made by the N-terminal halves of the two α3 helices of the RTP dimer, which contact two successive major grooves on the same face of the DNA. The positioning of the RTP dimer on the DNA and the knowledge of the centre of symmetry within the two dimer-binding sites has allowed a model of the complete RTP-*Ter* complex to be generated (Smith et al., 1996; Wilce et al., 2001). The model suggests that direct contact between the two adjacent RTP dimers bound at the overlapping A and B sites can occur while they are bound to approximately opposite sides of the DNA double helix, potentially explaining the highly cooperative binding observed in full-length terminator complexes. A structure of the complete functional RTP-*Ter* complex has not yet been obtained; this would greatly assist explanation of the mechanism by which polar replication fork arrest is achieved. Nevertheless, it is considered that the cooperative interaction between RTP dimers confers activity to the B-site end of the whole RTP-*Ter* complex, and the differential binding affinity and strand-asymmetry differences of RTP bound to the A and B sites, are important in establishing the functional polarity of the RTP-*Ter* complex (Duggin et al., 1999; Kralicek et al., 1997; Langley et al., 1993; Smith et al., 1996). While there was generally a correlation between RTP-*Ter* binding affinity and fork arrest efficiency, some mutant *Ter* sites do not strictly conform to this correlation, suggesting that the conformation of RTP-*Ter* has a meaningful role in the mechanism of fork arrest (Duggin et al., 2005). It was further suggested that the chock-like shape of RTP on the DNA could assist in wedging the advancing replisome. As a test of this hypothesis, peptides of differing size were attached to the C-terminus of RTP, placing them away from the DNA-binding site and in a position that was expected to come into contact with the advancing replisome (see Figure 2b) (Duggin, 2006). These RTP fusions had unaltered *Ter* DNA binding affinity, but showed fork arrest activity that reduced with increasing size of the attached peptide. This suggested that the replisome could remove the larger RTP-peptide fusions more easily from *Ter* DNA, and was taken to

be most consistent with the molecular chock model. Substantive evidence for specific interactions between RTP and the replisome is still lacking, since RTP point mutants examined so far had normal function or are defective in DNA binding (Duggin et al., 1999; Griffiths et al., 1998; Manna et al., 1996). Future studies should gain an understanding of the structure and dynamics of the whole RTP complex with forked DNA and *B. subtilis* replisome components.

Fork arrest at other sites

Fork arrest in *B. subtilis* also occurs at sites within 200 kb of *oriC* under conditions that induce the stringent response. The delay to replication under these nutrient-deprived conditions acts as a type of replication checkpoint, concurrent with other metabolic changes associated with the stringent response. The nature of the *B. subtilis* replication checkpoint differs markedly from *E. coli*, in which initiation of DNA replication at *oriC* is inhibited during the stringent response (Schreiber *et al.*, 1995). In *B. subtilis*, possibly three of the so-called STer sites exist to the left of *oriC* and two to the right. The second STer site to the left of *oriC*, named LSTer2, has been partially characterized (Autret et al., 1999). It was found that both RTP and the stringent response regulator protein RelA were required *in vivo* for fork-arrest activity at a segment identified to contain LSTer2. Also, the activation of fork blocking at LSTer2 depended on induction of stringent response conditions (Autret et al., 1999; Gautam and Bastia, 2001). Upon examination, the chromosomal segment containing LSTer2 was found to contain a sequence exhibiting similarity to the consensus B site of the terminus-region terminators. Significantly, specific deletion of the B-like site abolished stringent fork-arrest activity on the cloned fragment containing LSTer2 (Autret et al., 1999). The B-like sequence was found to bind purified RTP *in vitro*, but only one RTP dimer bound efficiently to the DNA fragments examined (Autret et al., 1999; Gautam and Bastia, 2001). Although these findings suggest an alternative make-up of the stringent and terminus-region terminators, it remains to be seen what factors are directly responsible for the regulation of stringent termination.

Fork arrest can also occur as an “unplanned” event, caused by collision of the replisome with other DNA-binding proteins or by encounter of DNA damage on the template. These tend to be stochastic events that could potentially take place at any position on the chromosome. In *E. coli* it has been shown that the major cause of replisome stalling is encounter with proteins bound to the template DNA, and of these RNA polymerase is the major culprit (Gupta et al., 2013). The accessory helicase Rep is travelling with replisomes and aids in displacing proteins if a replisome gets blocked (Guy et al., 2009). In the absence of Rep, UvrD may aid in this process, or may be required to resolve recombination intermediates that result from replisome collapse (Gupta et al., 2013; Petit and Ehrlich, 2002). PcrA likely plays a similar role in Gram positive bacteria (Naqvi et al., 2003). Genomes of bacteria have evolved to favour co-directionality of transcription with replication to avoid the potentially damaging head-on collisions that could otherwise occur (Rudolph et al., 2007).

Proteins bound to DNA have been utilised to artificially block DNA replication forks and are useful tools for examining chromosome biology; these range from introduction of ectopic *Ter* sites to addition of arrays of transcription repressor binding sites into the chromosome (Bidnenko et al., 2002; Payne et al., 2006; Possoz et al., 2006). When an ectopic highly efficient *Ter* site was placed roughly halfway round one of the chromosome arms to prevent replication proceeding further toward the terminus, cell survival was found to be dependent on RecA (Bidnenko et al., 2002). It was, therefore, proposed that a second fork from *oriC* collides with the stalled fork from behind and produces a double strand break that is repaired by homologous recombination. This repair also displaces Tus-*Ter* allowing replication to proceed past this point, although the precise mechanism for this is unknown. Fluorescently tagged transcriptional repressor proteins have been widely used to bind to arrays of their cognate binding sites to mark specific chromosomal loci to study chromosome dynamics in many organisms (Lau et al., 2003; Matzke et al., 2005; Straight et al., 1996). With a sufficiently large array of protein-DNA sites in tandem repeat, overproduction of the repressor protein leads to a replication roadblock that cannot be overcome (Possoz et al., 2006). Replication forks blocked at these repressor arrays have a half-life of 3–5 minutes, indicating that any long-lived unresolved protein block is a potential site for replisome dissociation (Mettrick and Grainge, 2016).

Another artificial situation is the overproduction of Tus. This activates additional weaker matches to the *Ter* site consensus in the *E. coli* chromosome, some of which are within reading frames and are considered unlikely to have evolved specifically as *Ter* sites (Duggin and Bell, 2009). Some of these sites are orientated in the blocking orientation of *oriC*-initiated replication and show a detectable pause in sensitive 2D gel electrophoresis assays when Tus is overproduced; these sites are not functional at physiological levels of Tus and were termed pseudo-terminators or *pTer* sites. By this definition, some of the outermost *Ter* sites previously assigned as part of replication fork trap due to their orientation in the genome would be considered *pTer* sites. The overproduction of Tus also caused some of the stronger chromosomal *Ter* sites, such as *TerG*, to show detectable pausing of replication forks approaching the normally permissive end of the complex (Duggin and Bell, 2009). It was considered that this might reflect an initial very brief pause by the replisome at the permissive end of Tus-*Ter*, that is normally undetectable in 2D gel assays, but with overproduced Tus, the *Ter* site can be re-bound by Tus at a rate that successfully competes for the DNA with the replisome, resulting in a weakly detectable pause.

Resolution of Daughter Chromosomes

After the fusion of two replication forks, some post-replicative events are frequently required so that complete separation of daughter chromosomes can be achieved. These events are the untangling of topologically interlinked daughter chromosomes (decatenation) and the resolution of dimeric chromosomes to monomeric daughter chromosomes that each newly forming cell eventually inherits.

During DNA replication the positive supercoiling that builds up as the replication fork progresses must be continuously removed. The primary enzyme involved in removing such superhelical stress is DNA gyrase, a type II topoisomerase (Kreuzer and Cozzarelli, 1979; Peebles et al., 1979). Despite the action of DNA gyrase, some so-called precatenation of the sister duplexes occurs as replication forks progress. The positive superhelical stress ahead of the fork can force the fork and associated proteins to rotate, thereby interwrapping the two daughter chromosomes. If not removed the precatentation causes the two chromosomes to remain topologically linked (catenated) after termination of DNA replication (Reyes-Lamothe et al., 2012). This barrier to chromosome segregation is largely solved by another type II topoisomerase, topoisomerase IV, which specifically resolves these catenanes (Espeli et al., 2003; Hojgaard et al., 1999; Reyes-Lamothe et al., 2012). This enzyme transiently cuts both strands of one daughter chromosome and then passes DNA from the other through the resulting gap before rejoining, thus removing an interlinkage.

The other obstacle to the separation of the two daughter chromosomes is a chromosome dimer—the result of an odd number of homologous recombination events between the two duplicated portions of the replicating chromosome. Homologous recombination is a significant by-product of the reactions needed to restart any replication fork that has stalled undesirably during DNA replication (Cox, 2001). This can eventually lead to the formation of a chromosome dimer. Both the *E. coli* and *B. subtilis* chromosomes contain a special recombination site, located within the terminus region, which allows the resolution of the chromosome dimers that might have formed; in *E. coli* it is close to the innermost *Ter* site *TerC*, while in *B. subtilis* it is adjacent to *TerVIII*. The site is named *dif* (deletion-induced filamentation) because its deletion leads to a filamentation phenotype as a result of the cell's inability to partition the joined chromosomes and complete cell division (the rod-shaped cells continue to grow lengthwise and form long filaments that eventually die) (Kuempel et al., 1991). The two copies of *dif* present in a chromosome dimer are juxtaposed during the late stages of chromosome segregation, which allows DNA strand exchange and chromosome dimer resolution into monomers (Barre et al., 2001; Reyes-Lamothe et al., 2012).

Several factors are required for functioning of the *dif* site. *dif* is composed of a 28-bp core sequence that acts as a binding site for the site-specific recombinases XerC and XerD (named CodV and RipX respectively in *B. subtilis*). In *E. coli*, chromosome dimer resolution at *dif* also requires the membrane-anchored FtsK, a large multifunctional protein (Grainge, 2013). FtsK is anchored at the division septum and loads onto polar sequences called KOPS (FtsK orienting polar sequences). These sequences are oriented towards the *dif* resolution site, which allows FtsK to distribute chromosomal DNA to either side of the septum, thereby preventing chromosome 'guillotining' (Bigot et al., 2005). Translocation of FtsK eventually will bring the *dif* site to the septal region (Barre et al., 2001). FtsK acts on the XerCD-*dif* complex inside the cell during the concomitant dimer resolution and cell division events. It directs the sequential activity of XerD, then of XerC, which imparts directionality to the recombination event

to resolve chromosome dimers to monomers effectively (and prevent formation of a dimer from two monomers) (Aussel et al., 2002; Barre et al., 2001; Grainge et al., 2011). In *B. subtilis*, there are two FtsK homologues; the DNA motor proteins SpoIIIE and SftA appear to work synergistically in clearing chromosomal DNA from the site of septation and in chromosome dimer resolution, although the mechanistic details remain less well defined than in *E. coli* (Biller and Burkholder, 2009; Kaimer et al., 2009; Kaimer et al., 2011).

Archaea with circular chromosomes are also expected to generate interlinked and dimer chromosomes that require resolution. While archaea may not contain specific fork traps (or are yet to be discovered), they contain Xer-*dif* dimer resolution systems homologous to bacteria (Cortez et al., 2010; Duggin et al., 2011). Some of the *dif* sites of archaea are not located in the expected terminus region, and this may reflect a very different mode of chromosome segregation and division control in these archaea compared to bacteria.

Physiological Roles of the Replication Fork Trap

The defined architecture of the *E. coli* chromosome with a single replication origin and a fork trap directly opposite dictates that the majority of replication fork fusion events will take place within the innermost *Ter* sites, even if one fork was to be delayed by obstacles such as protein-DNA complexes, DNA secondary structures or DNA lesions (cf Figures 1a & 4a). In *E. coli, TerC*, one of the innermost *Ter* sites, is located almost directly opposite *oriC*, while the second innermost *Ter* site, *TerA*, is 270 kb away. However, replication forks do not meet systematically at *Ter*/Tus complexes, as shown by early labelling experiments (Bouche et al., 1982) and more recently by high-resolution replication profiles, which allow quantification of the relative copy number of chromosomal areas. The majority of forks fuse in wild type *E. coli* cells close to the arithmetic mid-point, and the same region where fork fusions is observed both in the presence and absence of a functional fork trap (Dimude et al., 2016; Ivanova et al., 2015; Rudolph et al., 2013), suggesting that both forks normally move with similar speeds and, therefore, fuse freely within the region of the innermost *Ter* sites. The fork trap will come into play if one of the two forks is delayed on its way to the terminus, and 2D DNA gel electrophoresis, which can visualise replication intermediates such as forks paused at a *Ter*/Tus complex, showed that in normally growing wild type *E. coli* cells forks indeed arrest occasionally at *Ter*/Tus complexes. The strongest signal was observed at *TerC*, as might be expected given its proximity to the natural fusion site, followed by *TerA* (Duggin and Bell, 2009; Maduike et al., 2014).

What might be the physiological role of the replication fork trap? It has been suggested that it maintains the co-directionality of transcription and replication in the terminus. Both in *B. subtilis* and *E. coli* the vast majority of highly transcribed genes, such as *rrn* operons encoding ribosomal RNA, as well as genes encoding ribosomal proteins, are transcribed co-directionally with the direction of replication

(Figure 1) (Brewer, 1988; McLean et al., 1998). Indeed, there is ample evidence that head-on collisions of replication and transcription complexes pose a significant challenge to cells (Kim and Jinks-Robertson, 2012; McGlynn et al., 2012; Merrikh et al., 2012; Rudolph et al., 2007), especially if they occur at highly-transcribed genes such as *rrn* operons (Boubakri et al., 2010; De Septenville et al., 2012; Ivanova et al., 2015; Srivatsan et al., 2010; Wang et al., 2007). The fork trap would prevent clashes between replication and transcription by ensuring that replication forks from one replichore are not allowed to proceed into the opposite replichore (Brewer, 1988; Rudolph et al., 2007).

However, the situation is complex. While in *E. coli* over 90% of highly transcribed genes replication and transcription proceed co-directionally, genome-wide co-directionality is only just above average (55%) (Brewer, 1988; McLean et al., 1998). All *rrn* operons and the vast majority of genes encoding for ribosomal proteins are located in the origin-proximal half of the chromosome (Dimude et al., 2016; Jin et al., 2012). Thus, forks escaping the termination area would have to replicate at least another ¼ of the chromosome (1 Mbp) before reaching any of these highly-transcribed genes, which would only happen if the other fork is arrested for a considerable period. tRNA genes, also highly transcribed under fast growth conditions and more globally distributed throughout the chromosome, are mostly oriented co-directional in the origin-proximal half of the chromosome. Surprisingly, in the origin-distal half, orientation is much more heterogeneous, with a mild bias towards the head-on orientation for replication coming from *oriC* (Dimude et al., 2016). Thus, replication escaping the termination area might in fact encounter fewer problems than forks coming from *oriC*. There is little doubt that head-on collisions between replication and transcription can be very problematic for cells, but given the chromosome organisation it is debatable whether avoiding clashes between replication and highly transcribed areas are the main purpose of the terminus region in *E. coli*. On the other hand, *B. subtilis* shows a stronger co-directionality of replication and transcription.

Replication proceeding into the opposite replichore can be studied in cells containing ectopic replication origins in addition to *oriC*. In cells that carry an ectopic replication origin called *oriZ* (Figure 4b), located at the *lacZ* region, roughly ½ way round the clockwise replichore, replication of the chromosome becomes asymmetric (Ivanova et al., 2015; Wang et al., 2011). Replication initiating at *oriC* and proceeding counter-clockwise will have to replicate ½ of the chromosome, while forks initiated at *oriZ* and proceeding clockwise will get blocked at *Ter*/Tus complexes after replicating only ¼ of the chromosome (Figure 4b, green arrows). Upon deletion of *tus* the forks from *oriZ* will be able to proceed into the opposite replichore in an orientation opposite to normal (Figure 4c, blue arrows). If forks travelling in an orientation opposite to normal were delayed due to head-on collisions with transcribing RNA polymerase complexes, the reduced fork speed should shift the fork fusion point away from the arithmetic mid-point (mid-way between *oriC* and *oriZ*) towards the termination area (Figure 4c, grey arrows). Similar experiments in *Δtus* cells with an ectopic origin called *oriX* in the opposite replichore showed a fork fusion point essentially located at the arithmetic mid-point between *oriC* and

oriX, confirming that the speed of forks coming from *oriC* and forks escaping the termination area is at least similar (Dimude et al., 2018). Thus forks escaping the termination area appear to proceed with few problems, supporting the idea that avoiding replication-transcription clashes is unlikely to be the main purpose of the termination area.

A termination area forming an absolute fork trap actually poses a serious threat to cells; if one fork is very delayed at an obstacle, replication cannot be completed by the opposite fork because it will get blocked in the termination area (Figure 4a). Any stalled fork will have to be restarted or the cell affected will be in danger of dying, explaining the prominent role of replication restart proteins such as PriA in bacteria, which aid the recruitment of replication fork components at sites of damage (Gabbai and Marians, 2010). This scenario can be simulated in cells that carry the ectopic replication origin *oriZ* but in which *oriC* is deleted. If the fork trap is active then ¾ of the chromosome has to be replicated by the fork proceeding counter-clockwise, as the opposite fork will be blocked by *Ter*/Tus complexes after replicating ¼ of the chromosome (Figure 4d i, green arrows). The doubling time of this construct was shown to be delayed to about twice that of normal wild type cells (40 *vs* 20 min) (Ivanova et al., 2015). The inactivation of the replication fork trap by deleting the *tus* gene partially suppressed the slow growth phenotype of *ΔoriC oriZ* cells (doubling time: 29 min), demonstrating that the replication fork trap indeed inhibits fast growth if one fork is stalled at an obstacle for a long period of time (Figure 4dii) (Dimude et al., 2016; Ivanova et al., 2015).

Are there other phenotypes associated with the replication fork trap that might shed light on its most important physiological role? Various experimental approaches have shown that over-replication can occur at fork fusion sites. For example, plasmid R1 is replicated by a single replication fork moving uni-directionally around the plasmid until it is arrested at a single *Ter*/Tus complex (Nordstrom, 2006). In the absence of Tus protein an accumulation of complex branched DNA structures, plasmid multimers and rolling circle replication intermediates was observed (Krabbe et al., 1997). It was suggested that the helicase of the replication fork, upon reaching the already replicated area, might displace the existing nascent ends, which will generate intermediates that can serve as substrates for the continuation of replication (Krabbe et al., 1997). Similarly, *in vitro* studies had shown before that Tus prevented over-replication of a circular DNA containing a fork trap *in vitro* (Hiasa and Marians, 1994), lending support to a possible function of the bacterial replication fork trap in preventing over-replication.

A low level of chromosomal over-replication was also observed in the absence of Tus in otherwise wild type *E. coli* cells. This over-replication was exacerbated if additional mutations were added, such as point mutants in the *polA* gene, which encodes for polymerase I, a polymerase involved in DNA damage repair and Okazaki fragment maturation (Kurth and O'Donnell, 2009), suggesting one of the roles of Pol I might be to help bring DNA replication to a successful conclusion in the terminus region (Markovitz, 2005). Similarly, the absence of RTP in *B. subtilis* leads to phenotypes suggesting that an

increased number of chromosomal dimers are formed, which might also be the result of problems with terminating DNA replication accurately (Duggin et al., 2008; Lemon et al., 2001).

An even stronger effect of a lack of Tus protein was found more recently in cells lacking RecG helicase. The analysis of replication profiles in *ΔrecG* cells demonstrated a peak of over-replication in the termination area (Figure 5a) (Dimude et al., 2015; Rudolph et al., 2013; Wendel et al., 2014). Inactivation of the replication fork trap by deletion of *tus* allowed the observed over-replication to extend beyond the termination area in both directions. The forks which progress in a direction opposite to normal will suffer from head-on collisions with transcribing RNA polymerase complexes, especially at highly transcribed *rrn* operons, as described above. This can be alleviated by a point mutation called *rpo**, which destabilises ternary RNA polymerase complexes (Rudolph et al., 2007; Trautinger et al., 2005). *ΔrecG Δtus rpo** cells can survive the deletion of the entire *oriC* (Figure 5b), and appear to replicate by multiple rounds of recombination-based over-replication. Survival depends critically on the absence of Tus protein and is one of the strongest phenotypes of *Δtus* cells reported so far (Dimude et al., 2015; Rudolph et al., 2013). *ΔrecG Δtus rpo** cells in which *oriC* firing was inhibited showed an inverted replication profile where no sign of initiation is seen in the *oriC* area but, rather paradoxically, the highest levels of synthesis are observed in the termination area where forks normally fuse (Figure 5c) (Dimude et al., 2015; Rudolph et al., 2013). Thus, the level of over-replication in the absence of RecG is so substantial that it can sustain growth and division in the absence of *oriC* firing as long as the fork trap area is inactivated by deletion of *tus* and head-on replication-transcription conflicts are alleviated by an *rpo** point mutation.

What might be the reason for over-replication specifically in the termination area? One event that is specific for the termination area is the fusion of two replication forks (Figure 6a). We are only beginning to understand the events associated with two fusing forks, but the analysis of the over-replication seen in *ΔrecG* cells suggests that it might originate from DNA intermediates similar to those proposed for R1 replication (Dimude et al., 2016; Dimude et al., 2015; Krabbe et al., 1997; Lloyd and Rudolph, 2016; Rudolph et al., 2013). Upon fusion of two replication forks the helicase of one fork could displace leading strand of the opposing fork, resulting in the formation of a 3' ssDNA flap structure (Figure 3b). 3' flaps are an excellent substrate for RecG helicase (Bianco, 2015; McGlynn et al., 2001; Tanaka and Masai, 2006) and, in its presence, would be rapidly processed. 3' flaps also could be degraded by 3' single-stranded exonucleases, an idea supported by the observation that cells lacking all major 3' exonucleases also show over-replication in the terminus area (Rudolph et al., 2010a; Wendel et al., 2014). In the absence of either RecG or 3' exonucleases a 3' flap is likely to persist for longer and can be processed by the main replication fork restart protein PriA, which will trigger the assembly of a new replication fork (Figure 6c). Progression of such a fork will result in the generation of a double-stranded DNA end. Any such DNA end will be rapidly processed by the homologous recombination proteins RecBCD and RecA, leading to a strand invasion (a so-called D-loop) (Dimude et al., 2016; Dimude et

al., 2015; Rudolph et al., 2010a; Rudolph et al., 2010b; Rudolph et al., 2009; Rudolph et al., 2013). A D-loop is another substrate for PriA, which might establish another replication fork that would move in the opposite direction (Figure 6d). Progression of newly established forks will normally be blocked by *Ter*/Tus complexes as they proceed towards *oriC*, explaining why over-replication in cells lacking RecG is tightly restricted to the termination area. A recent analysis shows that over-replication in the absence of RecG can also be triggered outside of the termination area if forks are forced to fuse in an ectopic location (Midgley-Smith et al., 2018), strongly supporting the idea that the over-replication observed is triggered by events associated with the fusion of two replication forks.

These data suggest that one role of the termination area might be to contain the over-replication triggered by replication forks fusions to a defined area where appropriate processing can take place. The fact that increased levels of over-replication in the termination area are observed in cells lacking a number of proteins such as RecG, 3' exonucleases, DNA polymerase I and RecD (Markovitz, 2005; Rudolph et al., 2013; Wendel et al., 2014) suggests that a surprising number of proteins might be involved in the processing of fork fusion intermediates. In addition, processing of fork fusion intermediates by homologous recombination proteins will increase the frequency of recombination events in the termination area. Both over-replication of already replicated DNA and increased recombination frequencies have been found to contribute to genomic instability (Alexander and Orr-Weaver, 2016; Blow and Gillespie, 2008; Finkel et al., 2007; Tomasetti et al., 2017), providing another explanation for the importance of the replication fork trap.

That the processing of such intermediates might be of vital importance is illustrated by the fact that the consequences in cells lacking both RecG and 3' exonucleases are so severe that they are inviable (Rudolph et al., 2010a). A similar lethality was observed for the combination of a *polA* mutation with a *recG* deletion (Hong et al., 1995; Upton et al., 2014; Zhang et al., 2010). In addition, in *ΔrecG* cells carrying the ectopic replication origin *oriZ* the over-replication in the termination area was vastly exacerbated (Figure 5d). Over-replication is still observed in *ΔrecG* cells lacking Tus terminator protein, highlighting that over-replication is not triggered at forks arrested at *Ter*/Tus complexes. However, both *in vitro* and *in vivo* data suggest that arrested forks have a limited half-life and are disassembled after a relatively short period of time (Marians et al., 1998; McGlynn and Guy, 2008; Mettrick and Grainge, 2016). Thus, in *ΔrecG* cells in which replication forks are stalled at *Ter*/Tus complexes for an extended period of time, at least some forks will be disassembled. A collision event between a replisome and a partially disassembled fork might be particularly prone for triggering over-replication. In contrast, this risk will be considerably lower if one replisome is paused via the arrest of the helicase at a *Ter*/Tus complex, as both Tus and the arrested helicase will act as a "buffer" that stops progression of the helicase of the opposing fork. In wild type cells the stable arrest of replisomes at *Ter*/Tus complexes for some time might therefore be an important feature: it will actively prevent processing of stalled

forks while the second fork is likely to reach the termination area soon after (Midgley-Smith et al., 2018).

In line with the idea that the termination area might contain potentially harmful intermediates it was impossible to delete *oriC* in *oriZ*$^{+}$ *ΔrecG* cells. However, *oriZ*$^{+}$ *ΔoriC ΔrecG* was generated without much difficulty if the replication fork trap was inactivated by a *tus* deletion, confirming that in cells which replicate their chromosome asymmetrically from an ectopic origin things go catastrophically wrong, specifically in the termination area, in the absence of RecG (Midgley-Smith et al., 2018).

The comparison of the replication fork trap components in *E. coli* and *B. subtilis* have revealed no significant sequence or structural similarity, indicating that both systems might have evolved independently (Neylon et al., 2005), suggesting that a fork trap has a very important physiological function. But, so far the reported phenotypes for Δ*tus* and Δ*rtp* single mutants are rather mild. The hypothesis that the fusion of two replication forks, a necessary part of chromosome duplication, can have harmful consequences might offer not only an explanation for the importance of a fork trap, but might also at least in part explain why bacterial chromosomes are with almost no exception replicated from a single replication origin (Gao, 2015; Gao and Zhang, 2008). A chromosomal architecture with a single replication origin and a fork trap area, which per se defines two replichores, allows not only the co-orientation of replication and highly transcribed genes, but also minimises the number of replication fork fusion events to exactly one per cell cycle. It is tempting to speculate that the replication fork trap, rather than defining the location of fork fusion events, has evolved around the location where forks fuse naturally, and that over-replication can be safely contained in this region if the normal processing by proteins such as RecG, Pol I or 3' exonucleases fails for some reason.

Glossary

Circular chromosome
: The double-stranded bacterial DNA (deoxyribonucleic acid) chromosome forms one complete circle with no free ends.

Fork arrest
: Arrest (or pausing) of a replication fork at a defined location on the chromosome.

Helicase
: An enzyme that unwinds duplex DNA in an ATP-dependent manner.

Replication fork
: Junctions in partially replicated DNA where the newly synthesized daughter strands meet the unreplicated parental DNA.

Replication fork trap
: Two groups of terminators with opposite orientations arranged to trap replication forks in the terminus region of the chromosome.

Replicon
: A region of DNA replicated from a single origin of replication. It can be either a whole DNA molecule or part of one.

Replisome

A large multiprotein complex, located at the apex of the replication fork, that catalyses DNA replication.

Terminator

A DNA site that, upon binding a terminator protein, can bring about arrest (or pausing) of a replication fork.

Terminator protein

A protein that, upon binding to a specific DNA site known as a terminator, causes arrest (or pausing) of a replication fork.

Terminus region

A restricted and defined segment of a bacterial chromosome in which replication forks meet and fuse.

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Further Reading

Figure Legends

Figure 1. Arrangement of DNA replication terminators in the circular chromosomes of (A) *Escherichia coli* and (C) *Bacillus subtilis*. The two replication forks generated at the origin (*oriC*) move in opposite directions along the DNA and eventually approach one other and fuse within the terminus region diametrically opposed to *oriC*. The terminus region constitutes a replication fork trap in which the DNA terminators (denoted *Ter*) are arranged as two opposed groups, with the red terminators oriented to block movement of the clockwise replication fork and the blue terminators oriented to block the anticlockwise fork. Letters and roman numbers define *Ter* sites (*A* indicates the location of *TerA* in *E. coli*; *I* indicates the location of *TerI* in *B. subtilis*). The STer region of the *B. subtilis* chromosome contains additional terminator sites used only during the stringent response. The chromosomal locations for the origin, the *dif* chromosome dimer resolution site and the genes for the terminator proteins, Tus (terminus utilization substance) in *E. coli* and RTP (replication terminator protein) in *B. subtilis*, are marked. The location of *rrn* operons, which are highly transcribed particularly under fast growth conditions, are shown by green arrows, with the arrow pointing in the direction in which transcribing RNA polymerase molecules travel. **(B)** & **(D)** show consensus sequences for the *E. coli Ter* core sequence and the *B. subtilis* terminators. For *B. subtilis* the overlapping A and B sites are indicated.

Figure 2. Crystal structures of Terminator protein-DNA complexes. (A) Two crystal structures of the Tus-*Ter* complex of *E. coli* indicating the blocking and permissive ends of the complex (left, PDB 2i05), and in the "locked" conformation with DNA unwound at the blocking end and the C6 base of Ter DNA bound to its specific binding pocket in Tus (right,

PDB 2i06), which contributes significantly to the fork arrest activity of the complex. (B) Crystal structure of an RTP dimer in complex with the high-affinity half of *TerI* (the B site) (PDB 2efw). RTP can form a symmetric dimer in solution and recognizes the partial DNA sequence symmetry in each half site that makes up each functional *Ter* site in *B. subtilis*. However, the partial asymmetry of each half site causes the RTP monomers to adopt somewhat different conformations in the half-site complex ("wing-up" conformation, on the left-hand monomer, and "wing-down" conformation on the right-hand monomer as viewed), and this might play a specific role in establishing cooperativity of binding to the second low-affinity half-site (the A site), and optimizing contact with the oncoming replisome for its arrest. The cooperative binding of two dimers to each *Ter* site is essential for fork arrest activity. In the full complex, forks would be arrested when approaching from the right in the image shown. The structural basis for cooperative binding and how the whole complex interacts with the replisome are unknown features of interest.

Figure 3. Chromosome replication and cell growth in cells with one or two replication origins in the presence and absence of a replication fork trap. a) In the presence of a block to one replication fork on its way from *oriC* to the termination area the chromosome will remain under-replicated, as the second fork will be blocked by the *Ter*/Tus complexes in the termination area. **b)** Schematic representation of the replichore arrangement of an *E. coli* chromosome with an ectopic replication origin termed *oriZ* in the presence of a functional replication fork trap. *oriZ* indicates the integration of a duplication of the *oriC* sequence near the *lacZYA* operon (Wang et al., 2011). Directionality of replication and fork fusion locations are indicated by green arrows. **c)** Replichore parameters in the termination area of *E. coli* cells with two replication origins in the absence of a functional replication fork trap (Δ*tus*). If forks escaping the termination area proceed with a speed similar to forks coming from *oriC*, then the fusion point should be in the location indicated by the blue arrows. If forks escaping the termination area are slowed by an increased number of replication-transcription conflicts, then forks should fuse closer to the termination area, as indicated by the grey arrows. The experimental observation is, however, that the fork fusion point is located closer to *oriC* (green arrows), indicating that forks escaping the termination area potentially encounter fewer problems than forks coming from oriC (Ivanova et al., 2015). **d)** Schematic representation of the replichore arrangement of an *E. coli* chromosome replicating exclusively from an ectopic replication origin in the presence (d i) and absence (d ii) of a functional replication fork trap. Directionality of replication and approximate fork fusion locations are indicated by green and blue arrows in the presence and absence of a functional fork trap (*Δtus*), respectively.

Figure 4. Over-replication in the termination area in the absence of RecG helicase a) Replication profiles of *E. coli* cells in exponential phase. The number of reads (normalised against the reads for a stationary wild type control) is plotted against the chromosomal coordinate. Positions of *oriC* (green line) and primary *Ter* sites are shown above the plotted data with red and blue lines representing the left and right replichore as depicted in Figure 4A. The termination area between the innermost *Ter* sites is highlighted in light blue. Data were re-plotted from Rudolph *et al.* (2013). **b)** Growth of a *ΔrecG Δtus rpo** strain in which the entire *oriC* region is deleted. **c)** Marker frequency analysis of a *ΔrecG Δtus rpo** strain that carries a temperature-sensitive allele of the main replication initiator protein DnaA. The strain was grown at 42°C to inactivate DnaA(ts) and therefore prevent *oriC* from being active. Data were re-plotted from Rudolph *et al.* (2013). **d)** Marker frequency analysis of chromosome replication in a double origin strain in the presence and absence of RecG. Strains were grown at 37°C.

Figure 5. Schematic illustrating how replication fork fusions might trigger over-replication in the termination area and how this is normally prevented by proteins such as RecG and/or 3' exonucleases. Note that the formation of a 3' flap can occur at both forks. However, for simplicity the schematic shows only one such reaction. See text for further details.

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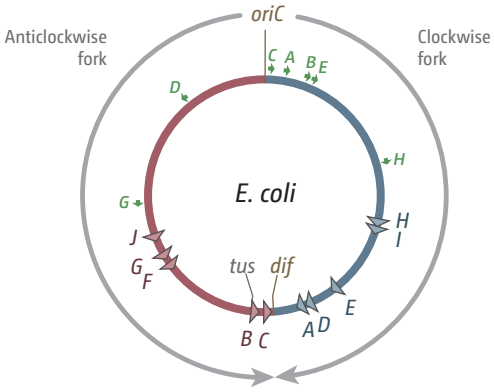
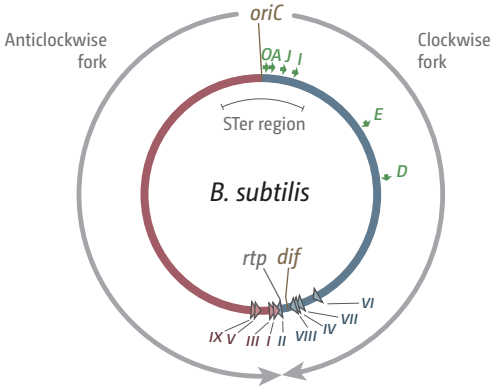
(A)



(B) *E. coli Ter* consensus sequence (central core)

$$\overset{6}{\text{G}}\underset{\text{G}}{\overset{\text{T}}{}}\text{A}\underset{\text{A}}{\overset{\text{T}}{}}\text{GTTGTAAC}\underset{\text{G}}{\overset{\text{T}}{}}\overset{18}{\text{A}}$$

(C)



(D) *B. subtilis ter* consensus sequence:

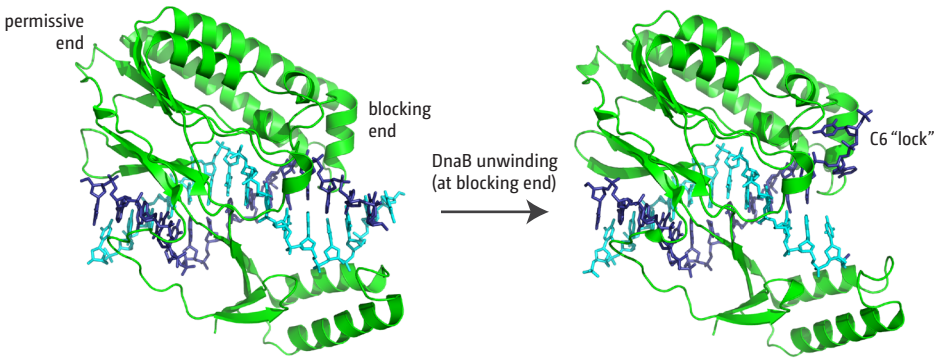
$$\overset{-2}{\text{NNACT}}\underset{\text{G}}{\overset{\text{A}}{}}\text{ANN}\underset{\text{G}}{\overset{\text{A}}{}}\text{N}\underset{\text{T T}}{\overset{\text{A C}}{}}\text{N}\underset{\text{G}}{\overset{\text{A}}{}}\text{TG}\underset{\text{A}}{\overset{\text{T}}{}}\text{AC}\underset{\text{C}}{\overset{\text{T}}{}}\text{AAATNTT}\underset{\text{T}}{\overset{\text{C}}{}}\overset{28}{\text{A}}$$

A site

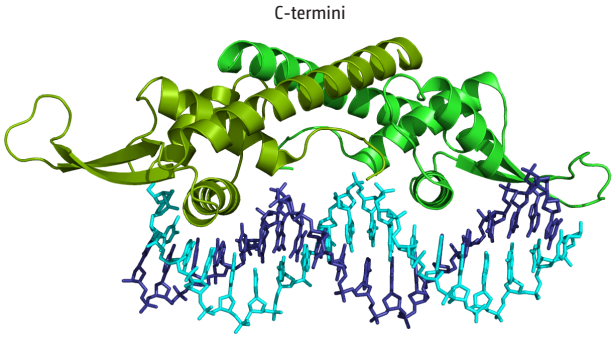
B site

Rudolph *et al.* Figure 1

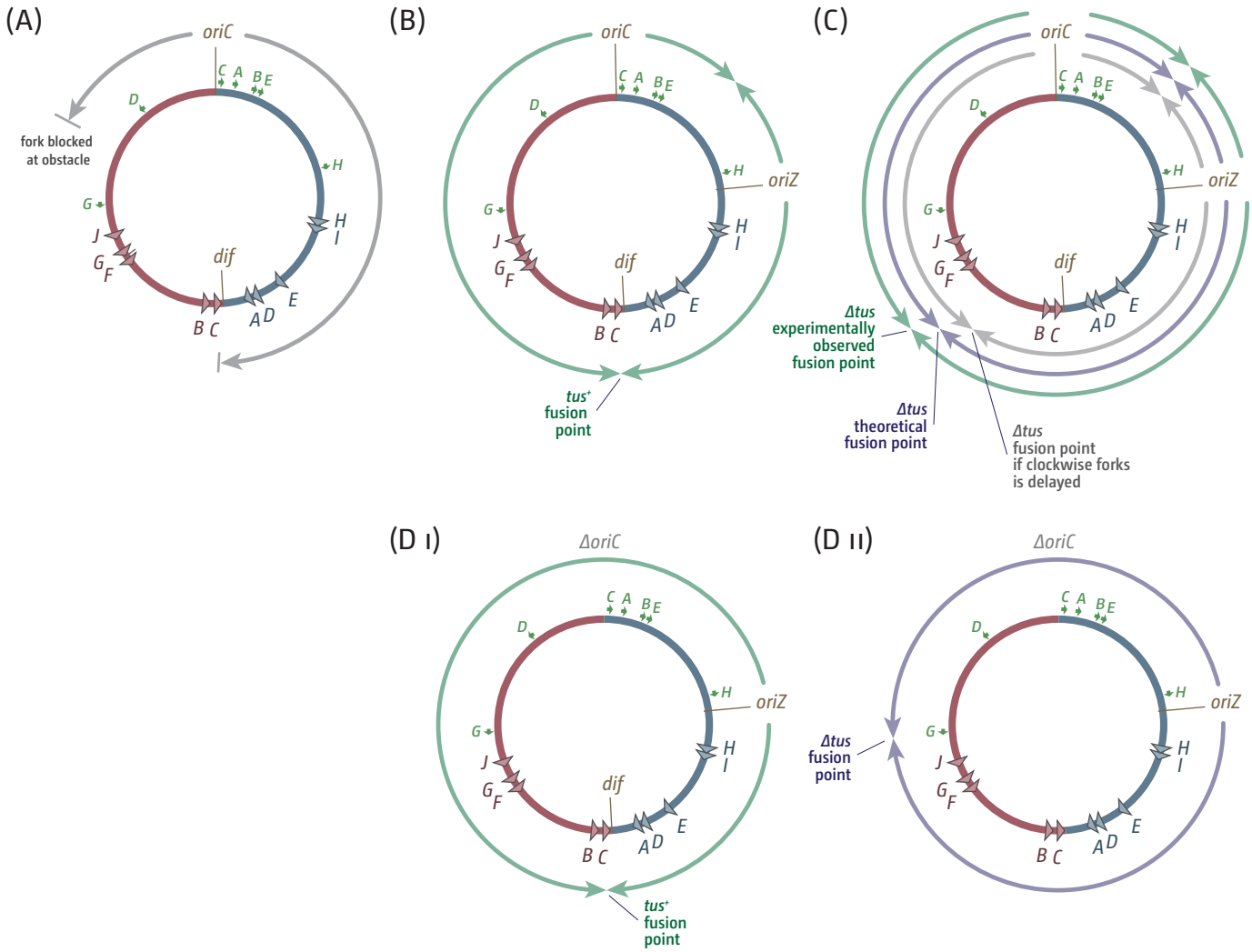
(A) *E. coli* Tus bound to *TerA* DNA



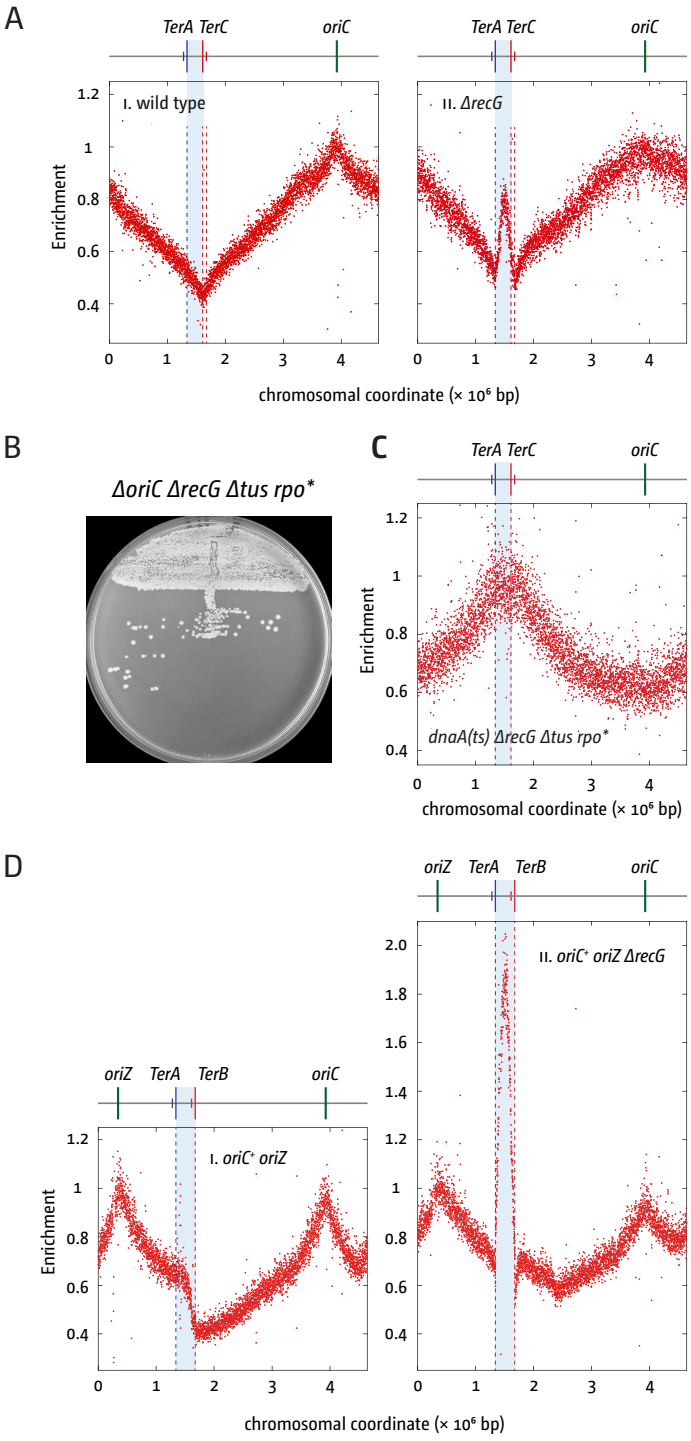
(B) *B. subtilis* RTP dimer bound to a *TerI* half-site DNA (the B-site)



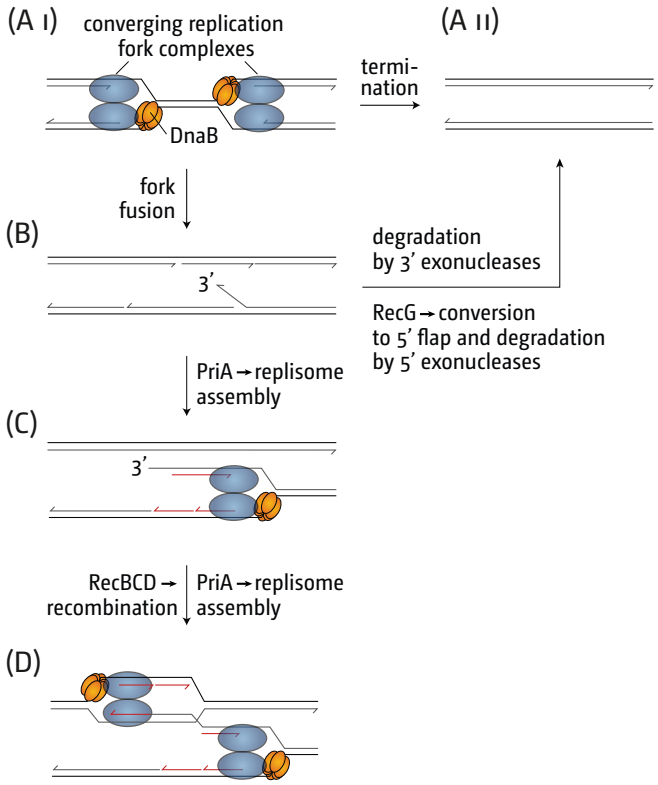
Rudolph *et al.* Figure 2



Rudolph *et al.* Figure 3



Rudolph *et al.* Figure 4



Rudolph *et al.* Figure 5