Helicases that underpin replication of protein-bound DNA in *Escherichia coli*

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Abstract
A pre-requisite for successful cell division in any organism is synthesis of an accurate copy of the genetic information needed for survival. This copying process is a mammoth task, given the amount of DNA that must be duplicated, but potential blocks to replication fork movement also pose a challenge for genome duplication. Damage to the template inhibits the replication machinery but proteins bound to the template such as RNA polymerases also present barriers to replication. This review discusses recent results from *Escherichia coli* that shed light on the roles of helicases in overcoming protein–DNA barriers to replication and that may illustrate fundamental aspects of how duplication of protein-bound DNA is underpinned in all organisms.

Introduction
Helicases play essential roles in both the actual process of DNA replication and the repair or resuscitation of blocked replication forks. This class of enzymes use the energy derived from hydrolysis of nucleoside triphosphates (usually ATP) to translocate along ss (single-stranded) or ds (double-stranded) nucleic acid and couple this translocation with disruption of hydrogen bonding between two nucleic acid strands, remodelling the nucleic acid structure [1]. This review focuses on the roles of helicases in genome duplication in *Escherichia coli*. This organism and its associated bacteriophages provide the best characterized systems with respect to mechanisms of DNA replication and of replication fork repair. Given that the substrate, DNA, has been retained as the carrier of genetic information in more complex organisms, *E. coli* also highlights some fundamental principles of genome duplication that likely apply to all organisms.

The replicative helicase in *E. coli*
Replication of DNA requires ss template to direct synthesis by DNA polymerases. Separation of the two parental strands is therefore the essential first step in DNA replication. The *E. coli* replicative helicase, DnaB, has a multimeric ring structure [2], as do other replicative helicases [3], and unwinds dsDNA by translocation along ssDNA with 5′→3′ polarity [4], placing DnaB on the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1). The toroidal structure of DnaB is central to the mechanism of unwinding. Unwinding proceeds by steric exclusion in which translocation of DnaB along the lagging strand template at replication forks (Figure 1).

When things go wrong
Many obstacles on the template DNA exist that can block replication fork movement. If these blocks are not removed or bypassed accurately, then mutation or incomplete genome duplication is the potentially disastrous consequence [7]. There are two main types of replicative block, damage to the DNA template and proteins bound to the template. DNA damage within one of the template strands prevents continued DNA synthesis by the relevant replicative polymerase, whereas cross-links between the two template strands inhibit template unwinding by the replicative helicase [8]. Proteins bound to the template also present barriers to replication since translocation by the replicative helicase must not only break hydrogen bonding between the parent DNA strands but also disrupt interactions between the template and any bound protein. Any high-affinity protein–DNA complex might inhibit fork movement *in vitro* [9], consistent with nucleoprotein complexes merely increasing the size of the energetic barrier faced by replisomes as they translocate along DNA. However, transcription complexes may present the most significant barriers to fork movement *in vivo* due to their abundance and very high affinity [9,10]. Additionally, damage within the transcribed DNA strand can inhibit catalysis by RNA polymerase, generating a very stable stalled transcription complex that may pose increased risks to replisome movement [11,12]. Thus damage to the template...
DNA may not only inhibit replisome movement directly, it may also enhance fork blockage by transcription complexes.

Removal of protein–DNA barriers usually requires only the disruption of non-covalent bonds, whereas repair of DNA damage requires breakage and formation of covalent bonds by excision repair enzymes [13]. Furthermore, damage in either the leading or the lagging strand template may generate extensive ssDNA gaps, unlike forks blocked by nucleoprotein complexes, creating a need for processing by recombination enzymes [14,15]. Processing of forks blocked by DNA damage may also be needed since the replisome may mask the damage from recognition by excision repair systems. Extensive processing of blocked forks, initiated possibly by RecG helicase via unwinding of the fork to form a four-stranded intermediate [12], implies that the replisome does not remain functional. Indeed, replisome reassembly is critical in tolerance of DNA damage, allowing replisome reloading on to processed fork structures or recombination intermediates away from oriC, the normal site of replication initiation [16]. PriA helicase and PriC provide overlapping mechanisms of replication restart in E. coli, with both facilitating DnaB reloading, and hence assembly of other replisome proteins, back on to inactivated forks and on to recombination intermediates formed as a result of blocked fork processing [17–20] (Figure 2). The physical and functional association of PriA with SSB protein, targeting PriA to SSB-bound ssDNA, may facilitate this replisome reassembly [21], but whether PriC is similarly targeted to DNA substrates is currently unknown.

Clearing proteins ahead of replication forks

The probable absence of extensive ssDNA gaps at forks blocked by protein–DNA complexes suggests that processing of the forked DNA by recombination enzymes may not be required. However, recombination enzymes are required in the presence of persistent protein–DNA blocks engineered into the chromosome of E. coli [22,23]. This requirement might reflect the collision of forks initiated later at oriC with the original blocked fork [24]. Recombination enzymes might also provide the means to reinitiate replication upstream of the block, in effect providing multiple opportunities for the fork to translocate through the block [23]. However, whether recombination enzymes are important in underpinning fork movement through native protein–DNA complexes is unclear.

Alternatively, clearance of a nucleoprotein complex ahead of a blocked but still functional fork might allow resumption of replication, given that blocked replisomes retain activity for at least a few minutes [25–27]. One potential clearance mechanism is the recruitment of accessory helicases

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**Figure 1 | Basic structure of replication forks in E. coli**
The DnaB hexameric ring encircles the lagging strand template, translocating in a 5′→3′ direction to effect unwinding of the parental duplex.

**Figure 2 | Mechanisms of replisome reassembly in E. coli**

(A) PriA helicase binds preferentially to branched DNA structures possessing a leading strand at or near the branch point. Binding of PriA results in assembly of a DnaB hexamer on to the lagging strand template via a series of interactions with other restart proteins. If this template strand is ss (i) then PriA helicase activity is not required. However, if the lagging strand arm is ds (ii) then PriA helicase activity is required to unwind the duplex and generate ssDNA on to which DnaB can be assembled. (B) PriC binds preferentially to branched structures lacking a leading strand near the branch point. If the lagging strand arm contains sufficient ssDNA on to which DnaB can be reloaded (i) then PriC can catalyse this reloading in the absence of Rep helicase. If the lagging strand arm is ds (ii) then Rep may be required to unwind this duplex and generate an ssDNA-binding site for DnaB reloading.
to forks in which translocation of the replicative helicase has been inhibited by a protein–DNA complex. Convincing evidence for accessory replicative helicases only arrived with analysis of the *Saccharomyces cerevisiae* Rrm3p helicase. Although not essential, and so not the main replicative helicase [28], Rrm3p promotes *S. cerevisiae* replisome movement through non-histone protein–DNA complexes [29,30].

**Rep**

Multiple lines of evidence had hinted at a possible accessory replicative helicase in *E. coli*. Rep. Replication forks travel at only half the speed in *rep* cells as compared with *rep*+ cells, which correlates with an increased amount of DNA and an increased number of replication forks in *rep* cells [31,32]. Although Rep does not act as the replicative helicase for the host chromosome, given the viability of *rep* mutants [32], the use of Rep by several bacteriophages as the viral replicative helicase indicated that Rep can act as part of a replication complex [33].

Further support for a Rep accessory function at the replisome was provided by the inability of strains lacking both Rep and the RecBCD helicase/exonuclease [34]. RecBCD processes dsDNA ends that might arise as a result of replication fork blockage and so *rep recBC* lethality might reflect increased fork blockage in the absence of Rep, generating an increased need for DNA end processing by RecBCD [35]. However, Rep has also been implicated in PriC-catalysed replication fork reassembly [19]. Assembly of DnaB, the first step in replisome reassembly, can occur only on ssDNA [4]. Since PriC is not a helicase, Rep may be required to unwind any duplex DNA on the lagging strand arm to facilitate PriC-directed replication fork restart [36] (Figure 2B, ii). This possible role in fork reloading complicates the interpretation of Rep function, as a defect in fork reassembly could create an increased requirement for RecBCD-promoted fork repair and might also explain the 2-fold decrease in replication fork speed in *rep* cells.

**Interplay between Rep and UvrD**

One reason for the difficulty in pinpointing Rep function *in vivo* was a functional redundancy between Rep and a second helicase that shares significant homology with Rep, UvrD. Although cells lacking either Rep or UvrD are viable, cells lacking both die under rapid growth conditions [37]. The molecular basis for this redundancy was revealed by screening of helicases for the promotion of fork movement through nucleoprotein blocks *in vitro*. Although either Rep or UvrD enhanced replisome movement, other tested *E. coli* helicases could not [38]. Simultaneously, both Rep and UvrD were shown to be needed for efficient replication across *rrn* operons that had been inverted on the chromosome such that their transcription was now head-on rather than co-directional with replication [39]. Such head-on collisions may be more inhibitory to fork movement that co-directional collisions [10,40]. This link between Rep, UvrD and transcription was also demonstrated by the identification of RNA polymerase mutations that destabilize transcription complexes and suppress *rep uvrD* lethality [38,39,41]. However, Rep and UvrD do not underpin fork movement specifically along transcribed DNA. *rep uvrD* cells carrying an RNA polymerase suppressor are hypersensitive to tandem *lac* repressor–operator complexes on the chromosome [38]. Thus Rep and UvrD can ameliorate such blocks regardless of their identity.

Both Rep and UvrD translocate 3′→5′ along ssDNA, suggesting that binding of ss leading strand template at the branch point of blocked forks might provide a common entry point for both helicases, allowing translocation ahead of the fork and dissociation of any blocking nucleoprotein complex [38] (Figure 3). Such a mechanism is supported by the analysis of helicases from other organisms that translocate either 5′→3′ or 3′→5′ along ssDNA. Promotion of fork movement along protein-bound DNA in *in vitro* and complementation of *rep uvrD* lethality *in vivo* was associated with 3′→5′ ssDNA translocation rather than 5′→3′ [38].

These results are consistent with both Rep and UvrD acting as accessory helicases to promote replication of protein-bound DNA. However, *rep* cells are more sensitive than *uvrD* cells to chromosomal inversions of *rrn* operons [39]. Furthermore, only Rep complements *rep uvrD* lethality when expressed at low levels [38]. This greater requirement for Rep as compared with UvrD also correlates with physical interaction of Rep, but not UvrD, with the replicative helicase DnaB [38]. This interaction is important for Rep-catalysed promotion of fork movement along protein-bound DNA *in vitro* and for *rep uvrD* complementation *in vivo* [38]. These results are consistent with Rep providing the accessory helicase activity required for replicating protein-bound DNA in wild-type cells, with UvrD providing this function only in the absence of Rep by virtue of the high intracellular concentration of UvrD.
DinG

A third helicase, DinG, might also be required for efficient movement of replisomes through highly transcribed operons oriented head-on to replication forks [39]. There is also a genetic interaction between dinG, rep and uvrD that indicates a role for DinG in underpinning genome duplication, possibly in the removal of transcription complexes [39]. Whether DinG can catalyse the displacement of nucleoprotein complexes ahead of forks is currently unclear. DinG translocates 5'→3' along ssDNA, [42], the opposite polarity to Rep and UvrD, indicating that DinG is unlikely to promote genome duplication in the same manner as Rep and UvrD. Complementation of the viability defect of dinG cells bearing an mrm inversion by overproduction of RNase H, a nuclease that specifically degrades RNA within RNA–DNA hybrids [43], also suggests DinG counters the deleterious effects of R-loops formed during transcription [39]. R-loop removal is essential for viability in E. coli [43] but there is little information concerning the impact of R-loops on replication fork movement.

The problem of replicating protein-bound DNA in other organisms

The S. cerevisiae accessory replicative helicase Rrm3p, like E. coli Rep, interacts with the replisome. However, interaction of Rrm3p is via one of the fork DNA polymerases [44] and/or the sliding clamp [45] rather than the replicative helicase. Thus, although localization of accessory helicases at the fork may be conserved, the interacting partner within the replisome might not be critical. Moreover, although both Rep and UvrD are 3'→5' helicases, Rrm3p translocates 5'→3' along ssDNA [46]. This may be related to the polarity of translocation of the primary replicative helicase. Although bacterial DnaB translocates 5'→3' along ssDNA [4], the eukaryotic MCM (minichromosome maintenance) complex translocates 3'→5' [3]. It may be that accessory helicase activity must be targeted to the template strand not bound by the replicative helicase. The differences between accessory helicases in E. coli and S. cerevisiae might therefore be revealing fundamental aspects of replicating protein-bound DNA in all organisms. However, these two organisms are currently the only systems in which accessory replicative helicases have been identified. Is the replication of protein-bound DNA a problem only encountered in bacteria and lower eukaryotes? The concept of fixed replication and transcription factories in higher eukaryotes is incompatible with there being direct conflict between genome duplication and gene expression. However, the idea of immobilized factories, especially with respect to transcription, is controversial [47]. Moreover, recent analyses of human mutation rates suggest that genome instability is enhanced where replication and transcription are oriented head-on [48]. More detailed analysis of the interplay between replication and transcription in higher organisms is required to determine whether all organisms face the problems uncovered in E. coli and S. cerevisiae.

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