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REVIEW ARTICLE

The Roles of Bacterial DNA Double-Strand Break Repair Proteins in Chromosomal DNA Replication

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One sentence summary: The close relationship between bacterial DNA replication and DNA double-strand break (DSB) repair is reviewed here, paying particular attention to recent research in Escherichia coli that has revealed two phenomena: DNA amplification of the chromosome terminus due to aberrant reactions in some DSB repair mutants and DNA loss in the chromosome terminus of DSB repair defective mutants.

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ABSTRACT

It is well established that DNA double-strand break (DSB) repair is required to underpin chromosomal DNA replication. Because DNA replication forks are prone to breakage, faithful DSB repair and correct replication fork restart are critically important. Cells, where the proteins required for DSB repair are absent or altered, display characteristic disturbances to genome replication. In this review, we analyze how bacterial DNA replication is perturbed in DSB repair mutant strains and explore the consequences of these perturbations for bacterial chromosome segregation and cell viability. Importantly, we look at how DNA replication and DSB repair processes are implicated in the striking recent observations of DNA amplification and DNA loss in the chromosome terminus of various mutant Escherichia coli strains. We also address the mutant conditions required for the remarkable ability to copy the entire E. coli genome, and to maintain cell viability, even in the absence of replication initiation from oriC, the unique origin of DNA replication in wild type cells. Furthermore, we discuss the models that have been proposed to explain these phenomena and assess how these models fit with the observed data, provide new insights and enhance our understanding of chromosomal replication and termination in bacteria.

Keywords: E. coli; DNA replication; DNA double-strand break repair; Homologous recombination
INTRODUCTION

In bacteria with circular chromosomes, DNA replication initiates at a fixed position called the origin (oriC in E. coli) and proceeds bidirectionally to reach the opposite side of the chromosome, where replication forks meet and replication terminates. In the Gram-negative bacterium E. coli, replication termination is constrained by 10 ter sites, which are bound by the protein Tus. Tus-bound ter sites create replication fork traps that allow forks to pass only in the permissive direction and block forks moving in a non-permissive direction. Replicating chromosomes start segregating into the two halves of the cell early during replication and final segregation occurs after completion of replication. However, the replication and segregation of chromosomes is far from being a simple phenomenon, as replisomes need to overcome several obstacles encountered while replicating the entire genome. These obstacles include, but are not limited to, fork breakage or stalling due to tightly bound proteins, DNA-protein crosslinks and replication-transcription conflicts.

Almost 50 years of intensive research have established profound roles of E. coli recombination proteins, required for the repair of DNA double-strand breaks (DSBs), in overcoming these obstacles to allow successful chromosome replication and to preserve genomic integrity (Skalka 1974; Capaldo and Barbour 1975a,b; Kuzminov 1995; Kuzminov 1999; Michel et al. 2018). Elegant genetic and biochemical experiments, carried out during this period, have revealed intricate details of their enzymatic functions, requirements in the cell, and mutant phenotypes. However, several interesting new observations, pertaining to the impact of DSB repair mutants on genome integrity and propagation, have been reported in the last decade and, accordingly, novel functions have been assigned to the proteins encoded (Rudolph et al. 2013; Wendel, Courcelle and Courcelle 2014; Azergilu et al. 2016; Dimude et al. 2016; Sinha et al. 2017; Dimude, Midgley-Smith and Rudolph 2018; Midgley-Smith, Dimude and Rudolph 2018; Sinha et al. 2018; Wendel et al. 2018; Midgley-Smith et al. 2019). Since these recent discoveries have sometimes led to apparently contradictory interpretations, it is necessary to discuss the experiments and conclusions together, while trying to understand the phenomena. It is also important to discuss these recent observations in the context of the wealth of information generated about these proteins in previous research and what is known about how recombination underpins DNA replication in other bacteria, bacteriophages and archaea. Here, we shall first recall the important functions of these proteins, their mutant phenotypes, and then discuss whether these known functions are able to explain the recent observations.

RECOMBINATIONAL REPAIR PROTEINS

RecBCD, RecA, RecFOR, RuvABC and their Corresponding Mutant Phenotypes

The RecBCD pathway is essential for homologous recombination mediated DSB repair in E. coli. Biochemically, the RecBCD protein complex is an exonuclease (ExoV) that has both 5´-3´ (in RecD) and 3´-5´ (in RecB) helicase activities (Dillingham and Kowalczykowski 2008). RecBCD can only bind to blunt or nearly blunt DNA double-strand ends (DSEs) and, owing to its helicase and exonuclease activities, it unwinds and cleaves DNA simultaneously. Encountering the regulatory ‘Crossover hotspot instigator’ (Chl) sequence (5´GCTGTTGGT 3´), RecBCD changes its property from nuclease to recombinase by generating a 3´ overhang onto which it loads RecA (Anderson and Kowalczykowski 1997; Dillingham and Kowalczykowski 2008; Handa et al. 2012; Taylor et al. 2014). RecA carries out homology searching in an intact homologous template (e.g. the sister chromatid), a Holliday junction is then formed, and this is resolved by RuvABC to form intact recombinants (Fig. 1) (Reviewed in (Kuzminov 1999; Dillingham and Kowalczykowski 2008; Michel and Leach 2012; Smith 2012)). Inactivation of either RecB or RecC abolishes the enzyme’s activity, whereas RecA inactivation primarily affects the nuclease activity of the enzyme complex, present in RecB. Additionally, recB and recC single mutants are recombination deficient whereas recD mutants are recombination proficient. Furthermore, recB, recC or ruvAB mutants are equally sensitive to exposure to DNA damaging agents such as of UV, ionizing radiation (such as X-rays, gamma rays etc.) or mitomycin C, while a recD mutant shows resistance similar to wild-type cells (Lloyd et al. 1984; McGlynn and Lloyd 2000; Dillingham and Kowalczykowski 2008). A recA mutant is extremely recombination deficient and sensitive to these DNA damaging agents owing to its role in both the RecBCD pathway and the alternative RecFOR pathway (Kuzminov 2011) (discussed below) (Table 1).

Viability

To discover the requirement of RecBCD in E. coli cells, grown under standard laboratory conditions (in the absence of external DNA damaging agents), an elegant experiment was performed to check colony forming abilities of equal numbers of cells (counted under the microscope) from wild type and mutants (Miranda and Kuzminov 2003). Interestingly, only ~30% of ΔrecBCD, ~50% of ΔrecA, and ~20% of ΔrecBCD ΔrecA cells could form colonies on plates, suggesting RecA-dependent and RecA-independent roles of the RecBCD complex (Table 1). Further experiments from this study showed that the RecA-independent requirement for RecBCD is dependent on its exonuclease activity. Similar losses of viability for these mutants were also reported in an earlier study (Capaldo, Ramsey and Barbour 1974). These observations led to the proposal that a subpopulation of growing E. coli cells experience the formation of replication dependent DSBs that can be reset by RecBCD-RecA mediated recombination or, in the absence of RecA, the broken chromosomal ends can be degraded by RecBCD exonuclease activity to generate intact chromosomes. Indeed, this proposal also explains earlier reports showing that ΔrecA cells exhibit an ‘asynchrony phenotype’ containing one or three chromosome forming units instead of the normal two or four, due to selective degradation of individual chromosomes (Skarstad and Boye 1993). Interestingly, it was also discovered that it is partially replicated chromosomes and not fully replicated chromosomes that are prone to RecBCD mediated degradation suggesting replication dependent DSB formation (Skarstad and Boye 1993). Additionally, ~5–10% of exponentially growing recA mutant cells were found to be anucleate due to ‘reckless’ DNA degradation and that degradation of the entire genome occurs, which is suppressed by inactivation of recBCD (Capaldo and Barbour 1975a,b; Zahradka et al. 2009).

Fork Reversal

RecA independent RecBCD essentiality was also observed in various replication mutant conditions such as rep, priA or mutants affected for different subunits of the holoenzyme DNA polymerase III (HE Pol III). In these mutant conditions, processing of arrested replication forks generates DNA double-strand ends (DSEs) via replication fork reversal (RFR). RecBCD is essential
Figure 1. RecBCD-dependent homologous recombinational repair of one-ended and two-ended DSBs. (i) A one-ended DSB is generated when a replication fork encounters a nick in a template strand, leading to replication fork collapse (a broken replication fork). (ii) Direct exposure to ionizing radiation generates a two-ended DSB. Both types of DSBs are unwound and cleaved by RecBCD using its helicase and nuclease activities. After recognizing the regulatory sequence Chi (5’-GCTGGTGG-3’), the properties of RecBCD enzyme change and a 3’-overhang terminated at a Chi sequence is generated. RecBCD then facilitates RecA loading onto this 3’-ssDNA overhang. The RecA-bound ssDNA searches for a homologous duplex DNA sequence, strand-exchange takes place to form a D-loop, followed by Holliday junction branch-migration and resolution by RuvABC proteins. PriA binds to the 3’ paired end(s), facilitates primosome assembly, and allows replication restart. Importantly, recombinational repair of a one-ended DSB leads to re-establishment of a replication fork, whereas two intact chromosomes are the products of two-ended DSB repair. Template strands and nascent strands are shown here as intact and dotted lines respectively. The leading-strand is denoted as a line with an arrowhead to mark the 3’-end.

to repair these reversed forks, either by RecA mediated recombination or RecA-independent degradation processes (recently reviewed in (Michel et al. 2018)). RFR also results from replication forks encountering DNA lesions (Fujiwara and Tatsumi 1976; Higgins, Kato and Strauss 1976; McGlynn and Lloyd 2000; Khan and Kuzminov 2012) and has been observed in bacteria growing under stress conditions (Schapiro, Libby and Fang 2003; Sinha et al. 2013).

RecBCD suppressors

Inactivation of the 3’-5’ exonuclease ExoI (encoded by the xonA gene, also known as sbcB for suppressor of recBC) was shown to suppress the recombination deficiency and DNA damage sensitivity of recBC mutant cells (Kushner et al. 1971; Kushner, Nagaishi and Clark 1972; Templin, Kushner and Clark 1972). Subsequently, it was discovered that these suppressor cells had
The sbcB sbcD mutant combination adversely affects the recombination efficiency of the RecBCD complex (in RecA− cells) suggesting that eliminating ExoI and SbcCd activities makes RecBCD activity inefficient (Thoms and Wackernagel 1998; Seigneur, Ehrlich and Michel 1999). Accordingly, the combination of sbcB and sbcD mutations (in RecBCD− cells) also inhibits the formation of anucleate cells and ‘reckless’ DNA degradation in a recA culture, similarly to the effect of a recB or recC mutation (Zahradka et al. 2009). Conversely to the activation of the RecF pathway described above, it was proposed that the action of SbcB and SbcCd proteins, to remove ssDNA overhangs at DSEs to make them blunt, allows efficient loading of RecBCD onto DNA and activation of the RecBCD pathway (Fig. 2A–D). This nucleolytic action of SbcB and SbcCd also becomes important for degradation of the entire chromosome or of a broken chromosomal arm in recA mutant cells. We suggest that frequent encounter of Chi sites on the genome of an sbcB sbcD mutant will inactivate RecBCD and leave a long tail of post-Chi 3′-ended ssDNA that needs to be degraded by 3′-5′ exonucleases to allow reloading of RecBCD (Fig. 2C–F). Therefore, in a sbcB sbcD strain, RecBCD cannot rescue cells by degrading a large part of a broken chromosome and it has to rely on RecA-loading for the recombination pathway, which is probably the reason for sbcB sbcD recA (RecBC−) strain being only ~20% viable (similarly to the recBCD recA strain) (Table 1; (Thoms and Wackernagel 1998; Thoms et al. 2008, Zahradka et al. 2009). This inactivation of RecBCD in an sbcB sbcD mutant is different from the inactivation of RecBCD by Chi in the presence of ExoI and SbcCd, which is dependent on RecA and is likely to be caused by titration of RecBCD molecules engaged in Chi-dependent DSB repair events (Kuzminov, Schabtach and Stahl 1994; Kuzminov and Stahl 1997; Thomas and Wackernagel 1998).

### Plasmid maintenance

The requirements for recombination proteins in plasmid multimerization, HMW (High-molecular-weight) DNA formation, and their impact on plasmid stability have been studied extensively. For θ-type plasmids, RecA was shown to be essential for HMW DNA formation suggesting that the HMW structures are formed by intermolecular homologous recombination, whereas for rolling-circle replicating (rcr) plasmids RecA facilitates HMW formation by protecting them from RecBCD mediated degradation (Cohen and Clark 1986; Silberstein and Cohen 1987; Niki et al. 1990; Dabert, Ehrlich and Gruss 1992; Dabert, Ehrlich and Gruss 1992; Kuzminov, Schabtach and Stahl 1994). Formation of HMW DNA by plasmid multimerization is responsible for the instability of plasmids due to its impact on copy number, resulting in improper segregation (Biek and Cohen 1986; Niki et al. 1988; Niki et al. 1990; Wendel,Courcelle and Courcelle 2014; Hamilton et al. 2019).

HMW DNA of mini-F and pBR322 plasmids is accumulated in recD and in recB sbcB mutants. Both of these strains lack exonuclease V activity but are recombination proficient, by RecBC in the former and by RecFOR in the latter strain (Cohen and Clark 1986; Niki et al. 1990). It was proposed that when a θ replicating plasmid is converted to rolling-circle replication either by endonuclease activity or by a fork encountering a nick, the newly generated tail could be degraded by RecBCD to facilitate formation of a monomer. However, in recombination proficient, exonuclease V deficient recD and recB sbcB mutants, the tail can recombine with an intact circular molecule to make a HMW multimer lacking a DSE (Nussbaum and Cohen 1988). Such molecules have been described as ‘pince-nez chromosomes’

### Table 1. Viabilities and recombination frequencies associated with recombination deficient mutants of E. coli

| Genotype                  | Viability (%) | Recombination frequency |
|---------------------------|--------------|-------------------------|
| Wild type                 | 100          | 1                       |
| ΔrecBC recD ΔrecA ΔrecBΔrecC | ~30          | 0.004                   |
| ΔrecA                      | ~50          | 0.00006                 |
| Δ recBC recA              | ~18          | 0.00006                 |
| ΔrecD                      | 100          | 1                       |
| Δ recA recD               | ~50          | 0.00004                 |
| Δ sbcB sbcD               | ~96          |                         |
| Δ recBC sbcB sbcC         | ~45          | 0.13                    |
| Δ recEF                    | ~12          | 0.002                   |
| Δ recBC sbcB sbcC recA    | ~19          |                         |
| Δ recF                     | ~71          | 0.5                     |
| Δ recA recF               | ~46          |                         |
| Δ recBC sbcD recF         | ~30          |                         |
| Δ recG                     | ~90          | 0.3                     |

*Value denotes conjugal recombination frequency.

(Clarke and Margules 1965; Horii and Clark 1973; Lovett, Luisi-Deluca and Kolodner 1988; Lloyd 1991; Miranda and Kuzminov 2003; Zahradka et al. 2006; Zahradka et al. 2009).
Action of ExoI and SbcCD nucleases facilitates RecBCD-mediated DSB repair by recombination in recA<sup>+</sup> cells and facilitates DNA degradation by RecBCD in recA<sup>−</sup> mutant cells. RecBCD enzyme can only bind and initiate unwinding and degradation of DNA that contains a blunt or nearly blunt DSE. Exonuclease I (the product of the sbcB gene, also known as xonA) and SbcCD both contribute to the generation of such ends that can bind RecBCD. This enables productive DSB repair by RecA (from A to D) or degradation of a broken chromosome, in the absence of RecA (from C to F). (Khan et al. 2016) and detected as exonuclease-resistant HMW DNA in a recBC sbcC sbcD mutant that is recombination proficient (recA<sup>−</sup> recF<sup>−</sup>) (Kusano, Nakayama and Nakayama 1989). An important point is that HMW DNA formation in wild type cells is dependent on the presence or absence of properly oriented Chi sites that will enhance intermolecular recombination by RecBCD and RecA proteins (Dabert, Ehrlich and Gruss 1992; Kuzminov, Schabtach and Stahl 1994; Smith 2012).

Plasmid multimerization and instability in a recBC mutant have recently been re-examined and an attempt has been made to extrapolate from the behavior of plasmids to that of the chromosome (Wendel, Courcelle and Courcelle 2014; Hamilton et al. 2019). However, fork breakage, which triggers sigma replication of chromosomes in a recBC mutant, occurs in 18% of cells on a 4.6 Mb chromosome (discussed later in this review). This corresponds to one break for every 25.5 Mb of DNA replicated. Clearly, such events occurring on a 5 kb plasmid present at 50–80 copies per cell would not be frequent enough to cause the observed growth impairment of a recBC mutant following transformation with a lambda-origin plasmid (Hamilton et al. 2019). Furthermore, the impact of nucleases on a 5 kb plasmid and on the chromosome are expected to be very different. A nuclease such as RecBCD, which acts at 1 kb per second, would totally degrade a 5 kb plasmid in 5 seconds, preventing any possibility of DSB repair, whereas in the chromosome it would on average take less than 5 seconds to reach a Chi site and initiate recombination, having degraded less than 0.1% of the genome.

**DNA DOUBLE-STRAND BREAK REPAIR IN GRAM-POSITIVE BACTERIA**

DSB repair processes in Gram-positive bacteria exhibit both similarities and differences to the processes in *E. coli*, described above. Nevertheless, the chronology of events following DSB generation is broadly similar (i.e. recognition of the broken ends and processing to generate a 3′ ssDNA tail, loading of RecA onto this ssDNA, followed by strand exchange, branch migration and resolution of Holliday junctions, and replication restart). However, despite having similar recombination machineries, there are structural and functional differences in the individual proteins encoded by *E. coli* and the Gram-positive bacterium *Bacillus subtilis*. 

Figure 2. Action of ExoI and SbcCD nucleases facilitates RecBCD-mediated DSB repair by recombination in recA<sup>+</sup> cells and facilitates DNA degradation by RecBCD in recA<sup>−</sup> mutant cells. RecBCD enzyme can only bind and initiate unwinding and degradation of DNA that contains a blunt or nearly blunt DSE. Exonuclease I (the product of the sbcB gene, also known as xonA) and SbcCD both contribute to the generation of such ends that can bind RecBCD. This enables productive DSB repair by RecA (from A to D) or degradation of a broken chromosome, in the absence of RecA (from C to F).
A well characterized feature of DSB repair in B. subtilis is the formation of a ‘repairer center’ (RC), which is marked by early association, and stabilization of DNA ends by RecN, a member of the SMC (structural maintenance of chromosomes) family of proteins (Ayora et al. 2011). This RC could be similar to the RC of eukaryotic systems, which facilitates repair of multiple DSBs at a single location, and is characterized by the presence of MRE11-Rad50 (also a member of the SMC family of proteins) (Lisby, Mortensen and Rothstein 2003). The end-processing reaction is carried out by the helicase-nuclease complex AddAB (a functional analogue of the RecBCD complex) to generate a 3' ssDNA overhang for RecA loading. The AddA subunit is a distant homologue of RecB, containing an N-terminal helicase domain and a C-terminal nuclease domain, whereas the AddB subunit has a second nuclease domain, at its C-terminus, not present in RecBCD. Similarly to the E. coli RecBCD complex, AddAB is regulated by a Chi site (5′ AGCGG 3′). However, in contrast to the RecBCD complex, there is no evidence of RecA loading by AddAB. Instead, RecOR (part of the RecFOR complex) has been shown to load RecA (Dillingham and Kowalczykowski 2008; Lenhart et al. 2012). RecA then carries out strand invasion and Holliday junctions are formed. Branch migration is mediated by RuvAB and Holliday junctions are resolved by the RecU endonuclease to reestablish replication forks.

Similarly to E. coli, another repair pathway includes the RecFOR proteins, RecQ helicase, and Rec exonuclease. In this pathway, the 5′ end at a DSB can be degraded by RecN, in concert with a RecQ helicase, and is followed by binding of SsbA protein to ssDNA. The RecFOR complex promotes the disassembly of SsbA and the loading of RecA. In contrast to E. coli, the Exol protein (SbcB) is absent in B. subtilis, while the RecFOR pathway is constitutively active. This implies that a multistep repair process, including multiple redundant protein functions, can promote DSB repair in B. subtilis (Ayora et al. 2011; Lenhart et al. 2012).

Inactivation of AddB and RecN, in the addAB recN double mutant, shows a synergistic loss of viability and increased sensitivity towards DNA damaging agents, similar to that of a recA single mutant (Sanchez et al. 2006). Fluorescently tagged RecQ and RecN were observed to colocalize with the replisome in normal growing cells, in the absence of any exogenous DNA damage, suggesting that their active roles may be in replication fork maintenance (Lecointe et al. 2007; Costes et al. 2010). Interestingly, the DNA damage response also determines the coupling between sporation and DNA replication, sporation being inhibited in a recA-dependent manner following DNA damage (Iretion and Grossman 1992). Additionally, RecA, RecO, AddAB, and RecU were shown to be required for replication restart, leading to efficient survival, following replication-transcription conflicts in B. subtilis (Million-Weaver, Samadpour and Merrick 2015).

The extremely radiation resistant Gram-positive bacterium Deinococcus radiodurans is a curious case as it lacks any functional homolog of AddAB (or RecBCD). However, it does contain homologs of the RecF pathway proteins including RecFOR, RecN, RecQ and RecA. Interestingly, RecQ is an essential protein for cell viability in this organism (Bentchikou et al. 2010). Deinococcus radiodurans can maintain 100% cell viability at a 10–20 fold higher radiation dose than is required to kill E. coli (Daly et al. 2004). This amazing survival potential is due to rapid reconstruction of an intact functional genome from scattered genomic fragments by an extended synthesis-dependent strand annealing (EDSDA) reaction and homologous recombination. EDSDA requires chromosomal fragments with overlapping sequences that are both used as primers and as template for complementary strand synthesis (Nassif et al. 1994; Zahradka et al. 2006b) and is facilitated by the existence of multiple copies of the genome in a single cell. As synthesis of an intact genome is dependent on RecFOR, RecA, and RecO proteins, recFOR or recO mutants are extremely radiation sensitive (Zahradka et al. 2006b; Bentchikou et al. 2010).

**STABLE DNA REPLICATION (SDR)**

The term Stable DNA Replication (SDR) was introduced to describe chromosome replication that is oriC and DnaA independent (Kogoma and Lark 1970; Kogoma 1978; Kogoma 1986; Kogoma 1997). E. coli mutants were isolated that can constitutively express SDR activity and this was termed ‘constitutive SDR (cSDR)’ (Kogoma 1978; Kogoma and von Meyenburg 1983). These cSDR mutants carried mutations in the rnhA gene, which encodes RNaseH1, an RNase that removes the RNA strand from RNA-DNA hybrids (R-loops). SDR can also be induced by repair of DSBs introduced either by UV treatment, thymine starvation, or by nalidixic acid treatment (Kogoma and Lark 1975; Kogoma, Torrey and Connaughton 1979; Magee and Kogoma 1990). This type of SDR was called ‘induced SDR (iSDR)’ and involves RecA-mediated recombination-dependent replication (Kogoma and Lark 1975; Kogoma 1986; Magee and Kogoma 1990; Asai and Kogoma 1994a). iSDR was measured as replication efficiency post DNA damage in the presence of rifampin and chloramphenicol to block oriC initiation (Magee et al. 1992). Both iSDR and cSDR require functional RecA protein (Lark and Lark 1979; Kogoma et al. 1985; Magee and Kogoma 1990).

**cSDR**

rnhA inactivation supports growth of E. coli cells deleted for oriC or inactivation of DnaA protein in minimal medium but not in rich medium (Kogoma and von Meyenburg 1983; Kogoma 1997). Contrary to iSDR, cSDR is sensitive to rifampin, which inhibits transcription. Thus, it was established that cSDR originates from R-loops, where the pairing of ssRNA within a dsDNA molecule is recognized and processed by PriA-mediated replication restart. Unlike iSDR, cSDR does not involve DSBs or SOS induction, thus it is not error prone. Using marker frequency analysis, five sites were shown to have higher copy number in rnhA mutant cells. They were named oriK sites. Interestingly, two of them were found to be near terC (de Massy, Fayet and Kogoma 1984).

**iSDR**

RecBCD- and RecA-mediated recombination is necessary for iSDR, indicating that D-loop mediated replication occurs, which has the potential to replicate a large part of the chromosome (Magee and Kogoma 1990; Magee et al. 1992; Asai et al. 1993; Asai and Kogoma 1974a,b,c; Asai, Bates and Kogoma 1994; Asai, Imai and Kogoma 1994). Surprisingly, marker frequency analysis using Southern hybridization revealed two specific regions with an increase in copy number during iSDR. One was in the oriC region (termed oriM1) and the other was in the terC region (termed oriM2) (Magee et al. 1992; Asai, Imai and Kogoma 1994). In these studies, iSDR was induced by either thymine starvation, UV irradiation or nalidixic acid treatment. Since these treatments are expected to generate DSBs across the genome (though later studies suggested thymine starvation causes breaks near oriC (Kuong and Kuzminov 2012; Khan and Kuzminov 2019)), D-loops are expected to be formed at distributed locations and this does not simply explain why Magee et al. 1992 observed a
copy number peak in the terC region. Importantly, the high copy number at terC was observed upon treatment with nalidixic acid regardless of the presence or absence of oriC, suggesting that it has nothing to do with forks originated at oriC.

It might be possible that D-loops and iSDR were generated across the genome but the high copy number was visible in the terC region due to blockage of forks at terC. Alternatively, the generation of DSBs across the genome and their repair might somehow induce increased copy number at oriM1 and oriM2. However, an attempt to map specific loci that can exhibit oriM1 and oriM2 activity in a heterologous system was not successful, suggesting that these increases in copy number are the direct outcome of random chromosome DSB generation and repair by RecBCD-RecA mediated homologous recombination and not due to any sequence-specific origin induction. This was supported by the observation that a recD mutation (which induces hyper-recombination) enhances iSDR after thymine starvation and nalidixic acid treatment (Magee & Kogoma 1990; Magee et al. 1994). However, experiments were not carried out to ascertain whether there is any specific origin induction in these cells. RecG is a DNA helicase that has branch migration activity and recG mutants are sensitive to DNA damaging agents (Storm et al. 1971; Lloyd and Buckman 1991; Kalman, Murphy and Cashel 1992; Lloyd and Sharples 1993). The recG mutation is also synthetically lethal with rnhA inactivation suggesting that RecG may play a role in R-loop removal (Hong, Cadwell and Kogoma 1995). Thus, continuous DNA synthesis observed in a recG mutant could either stem from persistent R-loops or from D-loops accumulated because of the absence of DNA branch migration activity, or both. Accordingly, a recG mutant exhibited SDR at a similar level to that of an rnhA mutant but only half of it was sensitive to rifampin. R-loop mediated SDR will be sensitive to rifampin, suggesting that at least the other half was dependent on D-loops. Indeed, half of the SDR was sensitive to recB mutation, which would block D-loop formation (Hong, Cadwell and Kogoma 1995). Contrary to the rnhA mutant, recG inactivation could not rescue cells devoid of oriC or DnaA, and since the recG mutant exhibits SDR at a similar level to that of the rnhA mutant (Hong, Cadwell and Kogoma 1995), it was postulated that the mode of SDR (origin usage and direction of replication) in a recG mutant may not be suitable to replicate the entire chromosome (Kogoma 1997). We shall discuss this further in section 6 of this review and see how this proposal proved to be correct, as two further mutations are required to make this possible.

PriA

PriA is required for replication restart pathways (Lee and Kornberg 1991; Nurse, Zavitz and Marians 1991; Michel and Sandler 2017; Michel et al. 2018). The priA null mutant is sick, grows slowly in minimal medium, does not grow in rich medium, and is completely inactive for SDR (Sandler, Samra and Clark 1996; Michel et al. 2018). However, PriA helicase activity is not essential for primosome assembly and PriA (K230R) (encoded by the priA300 allele), which is deficient in ATPase and helicase activity, still supports SDR initiation (Kogoma et al. 1996; Dimude et al. 2015). Remarkably, inactivation of PriA helicase activity (by the srgA or priA300 mutations) suppresses the DNA damage sensitivity of recC mutants (Al-Deib, Mahdi and Lloyd 1996; Jakstai and Lloyd 2003). This indicated that PriA and RecG have opposing roles and that the requirement for RecG protein in DSB repair may not be direct. Instead, RecG might prevent undesirable consequences of PriA helicase activity during DSB repair. It has been shown that RecG helps the correct loading of PriA and DnaB for replication restart (Tanaka and Masai 2006) and in the absence of RecG, PriA can load DnaB incorrectly causing replication to restart in the opposite direction (reverse-restart) (Azeroglu et al. 2016; Michel et al. 2018) (Fig. 3).

ORIGIN-INDEPENDENT DNA REPLICAION IN OTHER SYSTEMS

Recombination-dependent DNA replication (RDR) has been observed in several other systems and these can be thought of as analogous to SDR in E. coli. Furthermore, RDR can lead to genome replication, even without the need for replication initiation from a fixed origin of replication.

Bacteriophage T4 is the classic model system where RDR was originally proposed and studied (Luder and Mosig 1982; Kreuzer et al. 1988). T4 expresses a full set of its own recombination proteins at high level during T4 infections of E. coli. In fact, T4 uses D-loop formation as a major mode of DNA replication initiation during late stages of the lytic cycle (Kreuzer and Brister 2010; Liu and Morrical 2010). Initiation of early T4 DNA replication occurs at specific origins (oriF and oriG) by the formation of R-loops. Since the infecting T4 phage genome is linear and circularly permuted, when this origin-initiated replication reaches the DNA ends, randomly located ssDNA 3′ termini are generated. These 3′ ssDNA ends are then used by the recombinase UvX (recruited by UvY to polymerize on gp32-complexed ssDNA) to catalyze strand invasion, either into the other end of the same phage genome, which has the same sequence repeated, or into the middle of another phage DNA (facilitated by the circular permutation of the genome). Strand invasion is followed by D-loop mediated replication initiation and is thus responsible for net genome duplication in the late stages of lytic cycle (Luder and Mosig 1982; Kreuzer et al. 1995; Kreuzer and Brister 2010; Liu and Morrical 2010).

Origin independent replication has also been reported in the archaeon Haloferax volcanii. This archaeon can survive even after deletion of all four of its replication origins. Interestingly, the deletion of all four origins confers 7.5% faster growth rate than the wild type strain. Nonetheless, the absolute requirement for
Figure 3. Four models to explain terminus DNA amplification in a recG mutant. (i) Reverse-restart model: This model proposes concerted action of RecG and PriA in the re-establishment of a correctly oriented replication fork. According to this model, during broken replication fork repair and reassembly, the presence of RecG allows correct PriA-mediated loading of DnaB helicase on the lagging-strand template, potentiating correct replication restart (from a to d). In the absence of RecG, PriA is no longer correctly directed by RecG and can mistakenly load DnaB on the nascent lagging-strand (e). This initiates reverse-restart, via the assembly of a replication fork proceeding in the opposite direction. Meanwhile, PriA can still load DnaB in the correct location setting up a correctly oriented replication fork (the absence of RecG does not preclude normal DnaB loading, it simply permits incorrect loading) (f). The end of the ds-DNA flap that has been generated can invade and recombine with the intact chromosome (RecA-RecBCD mediated recombination). Thus, divergent forks are established causing DNA amplification (g). If, during recombination, PriA again loads DnaB incorrectly a new flap can be generated adding to the amplification (from g to e). If new breaks are generated at one or other of the Tus/ter sites, the cycle can be re-initiated adding to amplification (from g to a). (ii) 3′-flap model: This model proposes that merging of replication forks in the terminus generates a 3′-flap (from a to b). RecG binds to this 3′-flap and converts it into 5′-flap to be degraded by 5′-exonucleases to complete termination (from c to d). In the absence of RecG, the 3′-flap will be used by PriA for primosome assembly and restart of the replication fork (e). The end of the ds-DNA flap that has been generated will invade and recombine with the intact chromosome (RecA-RecBCD mediated recombination). Thus, bidirectional forks are established causing DNA amplification (f). (iii) Over-replication model: This model proposes that merging replication forks can pass each other, leading to amplification of the terminus region (from a to b). The over-replicated region is processed by RecG to make it available for degradation by RecBCD and other exonucleases. In the absence of recG or exonucleases, the over-replicated region persists. (iv) Fork-trap model: This model simply proposes that Tus/ter mediated trapping of multiple forks generated by stable DNA replication in a recG mutant leads to the observation of terminus DNA amplification (a-b). Yellow triangles represent the Tus/ter trap; the black circle represents the DnaB helicase. Template strands and nascent strands are shown here as intact and dotted lines respectively. The leading-strand is denoted as a line with an arrowhead representing the 3′-end.
the recombinase RadA indicates that D-loop/R-loop mediated replication initiation (similar to SDR in *E. coli*) is responsible for chromosomal replication and survival of the origin-deleted *H. volcanii* strain (AusianniKava and Allers 2017).

**RECENT OBSERVATIONS OF AMPLIFICATION AND DEGRADATION OF DNA IN THE TERMINUS OF *E. coli* MUTANTS**

In the last decade or so, a surge of new observations has emerged on the effects of *E. coli* DSB repair mutants on genome integrity and propagation, stimulated in large part by whole genome sequencing methods of marker frequency analysis (MFA). The two most prominent observations are amplification and loss of DNA in the terminus region. A large set of mutants exhibit terminus region amplification, whereas terminus region loss is specific to recBC mutants (Dimude et al. 2015; Ivanova et al. 2015; Dimude, Midgley-Smith and Rudolph 2018; Rudolph, Upton and Lloyd 2009; Rudolph et al. 2009, 2013; Wendel, Courcelle and Cournelle 2014; Courcelle et al. 2015; Sinha et al. 2017, 2018; Wendel et al. 2018; Raghunathan et al. 2019). We shall first discuss the evidence and mutant conditions for terminus amplification and then analyze DNA loss separately.

**DNA amplification of the chromosome terminus region**

Terminus DNA amplification has been reported in recG, dam, exo, rnhA and topA mutants. Given the diversity of these genes, we expect that this will not be an exclusive list of genes implicated in minimizing terminus region amplification. Recently, it has been shown that DSBR repair can stimulate terminus region amplification even in a wild type cell (White et al. 2020).

**Amplification in recG mutant**

When iSDR in the recG mutant was reinvestigated using pulsed field gel electrophoresis (PFGE), it was shown to occur over all the genome following UV irradiation (Rudolph, Upton and Lloyd 2009; Rudolph et al. 2009). This was satisfying as UV was expected to induce distributed DSBS (Khan and Kuzminov 2012), the repair of which could initiate randomly distributed D-loop mediated replication. In addition, when the ori and ter regions were examined by fluorescence microscopy, amplification of these two locations was confirmed upon iSDR, similarly to the previous description of oriM1 and oriM2 (Magee et al. 1992; Asai, Imai and Kogoma 1994, Rudolph, Upton and Lloyd 2009; Rudolph et al. 2009). Interestingly, recG inactivation delayed the recovery of UV irradiated cells. These cells remained elongated, had much higher numbers of both origin and terminus foci and were affected for chromosome segregation for a long time compared to wild type cells. Nonetheless, this phenotype was also suppressed (or highly reduced) by inactivation of PriA helicase activity (Rudolph, Upton and Lloyd 2009; Rudolph et al. 2009). Based on these data, a model was proposed to explain persistent high SDR activity in UV irradiated recG cells.

According to this model, induced replication forks would meet each other and the replisome of one fork may displace the 3´-end of the oncoming fork generating a 3´ flap. RecG, owing to its preference to unwind a fork substrate with a 5´ end at the fork junction, will bind and convert a 3´ flap to a 5´ flap thereby limiting PriA mediated re-replication in the opposite direction. The 5´ flap will be eliminated by a 5´-3´ ssDNA exonuclease.

In the absence of RecG, PriA mediated restart will further generate a DSE substrate for RecBCD-RecA mediated recombination and amplify the region adjacent to the point of fork collision (Rudolph, Upton and Lloyd 2009) (Fig. 3i). However, this model assumes PriA mediated restart from a 3´ flap, which has not been documented biochemically. Instead, it has been shown that PriA needs a 3´ end paired to the template to initiate replication (Heller and Marians 2005, Bhattacharyya et al. 2014, Michel and Sandier 2017). Therefore, PriA mediated restart from a 3´ flap remains hypothetical.

Using whole genome sequencing based MFA analysis, a striking observation was made. An exponentially growing recG mutant specifically amplifies the copy number of the terminus region, between terA and terB, even without any UV treatment (Rudolph et al. 2013). This amplification is abolished by inactivation of PriA helicase activity and by inactivation of recB suggesting SDR involvement. This SDR dependent amplification in a recG mutant allows cells to tolerate and survive the loss of DnaA if two further obstacles are removed: first, in a tus mutant outward directed forks can move beyond the terminus region; and second: in a rpoB‡ mutant, which destabilizes transcription complexes, forks can proceed round the chromosome (Rudolph et al. 2013). These results established that SDR mediated replication (including its requirement for RecBCD-RecA mediated DSBR repair) is efficient in replicating the entire chromosome but is normally limited by replication-transcription collisions, since most of the highly transcribed genes (e.g. rRNA operons) are co-directional with replication forks generated at oriC, whereas replication forks generated by SDR have a high chance of moving in the opposite direction (Rudolph et al. 2013; Dimude et al. 2015). Accordingly, a dnaA46 recG tus rpoB‡ strain (no replication initiation at oriC, no RecG, and no blockage by Tus or RNA polymerase of replication forks moving from terminus to origin) grows at the non-permissive temperature (42°C) and its MFA replication profile shows the highest peak at the terminus and the lowest at oriC, the opposite to a normal replication profile.

The same model was used to explain this terminus amplification observed in a recG mutant as had been proposed to explain high SDR occurring elsewhere on the chromosome upon UV irradiation (Rudolph, Upton and Lloyd 2009). Notably, it was proposed that, when two forks (normally originated at oriC) meet in the terminus region, they generate a 3´ flap and, in the absence of recG, this 3´ flap is used by PriA to restart new replication forks moving in opposite directions (Fig. 3i). Thus, it was suggested that fork collision has the potential to destabilize genomic integrity, while RecG prevents this by converting 3´ flaps into 5´ flaps at fork collision sites. The observation that inactivation of multiple 3´ ssDNA exonucleases (xseA xonA sbcCD) also gives a similar MFA replication profile of terminus amplification, despite the presence of RecG was used as further support for the model (Rudolph et al. 2013). However, as mentioned above, a 3´ flap is not a known substrate for PriA mediated replication restart. More importantly, the inverted MFA profile (highest at terminus and lowest at origin) cannot simply be explained by forks merging at the terminus, since forks do not originate from oriC at the non-permissive temperature in a strain with the dnaA46 mutation.

Alternatively, a reverse-restart model was proposed to explain the dependent amplification in the recG mutant (Azeroglu et al. 2016). According to this model, PriA can promote reverse-restart at a Tus/ter barrier in the absence of recG (Fig. 3i). This fork moving in the opposite direction will reach the opposite ter site and again a new reverse-restart fork will be assembled in the opposite direction. At each step a DSE will be generated that
can recombine to generate another fork. This back and forth replication is proposed to amplify the terminus region in recG cells. Support for this model was provided by RecA ChIP-seq data showing outward directed RecA binding at terA and terB sites, demonstrating the presence and location of the predicted DSEs (Azeroglu et al. 2016). Furthermore, DNA amplification in a recG mutant was observed at the site of DNA double-strand breakage in the lacZ locus, distant from the chromosome terminus (Azeroglu et al. 2016), raising the possibility that, in the absence of RecG, reverse-restart can potentially occur following attempted recombination at any chromosomal DSE. It was previously shown that forks blocked at ter sites are prone to the generation of DSEs (Horiiuchi et al. 1994).

Two further models have been proposed. In the third model, it was suggested that merging replications might cross each other and over-replicate the terminus region (Fig. 3iii) (Wendel, Courcelle and Courcelle 2014). In this model, RecG was hypothesized to reverse one of these forks generating a DSE that was degraded by RecBCD to remove the over-replication. In this way, a recG mutant would retain the over-replicated region. Finally, in the fourth model, it was suggested that SDR initiated at random locations resulted in terminus over-representation in MFA simply because of the nature of the Tus/ter trap that would not allow replication to proceed across the terminus to a Tus/ter block in two directions in a population of cells (Fig. 3iv) (Gowrishankar 2015).

Not all features of these models are mutually exclusive and some data fit some models and not others, while some data seem incompatible with all models as they currently stand. Several key observations need to be accommodated in our understanding: 1. One-ended DSEs accumulate at Tus/terA and Tus/terB sites in a recG mutant and these are the boundaries of the amplified region in a strain with an intact Tus/ter system (Azeroglu et al. 2016). 2. In a strain harboring two origins (oriC and oriZ), amplification is only seen at the terminus site although forks meet at two different places on the chromosome (Rudolph et al. 2013). 3.Terminus amplification is still visible (although reduced) in recG cells harboring a linear chromosome, where forks would never meet (Rudolph et al. 2013; Dimude et al. 2015). 4. The MFA profile of a dnaA46 recG tus rpo* strain shows a maximum in the terminus and a minimum in the oriC region. Observations 1. and 2. suggest that an interaction with the Tus/ter system plays some role in the DNA amplification observed in a recG mutant and this correlates with an elevated level of DSEs at these sites. Observation 3. suggests that the meeting of replication forks originating from oriC is not required for amplification in a recG mutant. Observation 4. suggests that there is something important about the terminus region that determines the initiation of DNA replication in a recG mutant even in the absence of a functioning Tus/ter system and in the absence of DNA-dependent initiation of replication at oriC.

Further investigation revealed that the amplification observed in a recG mutant is not dependent on R-loops, as overexpression of yeast RNaseH1 does not affect the viability of a dnaA46 recG tus rpo* strain (Dimude et al. 2015). All of these new and earlier observations clearly suggest that RecG keeps D-loops in check to prevent SDR. Exponentially growing E. coli cells are prone to suffer replication-dependent DSBs (Kuzminov 1995, 2001; Miranda and Kuzminov 2003; Michel et al. 2018). Repair of these DSBs will initiate D-loop mediated replication, which can be exacerbated in absence of RecG, perhaps by a reverse-restart mechanism and then by replication-transcription collisions; but consequences only become visible in the terminus region due to the presence of ter sites, which may contribute themselves to the amplification through stimulating further DNA breaks and driving a precise localization of amplified DNA, while replication-transcription collisions occur all over the chromosome.

**Amplification in dam mutant**

Recently the dam mutant was also shown to have amplification in the terminus region and to allow deletion of dnaA when combined with tus and rpo* mutations (Raghunathan et al. 2019). As for recG mutants, the priA300 allele abolished this survival advantage suggesting SDR. A crucial role of DinG protein in overcoming replication-transcription collisions was also reported when cells are replicating by SDR in a dam mutant. SDR initiated in a dam mutant is due to DSBs generated by the mismatch repair pathway, as inactivation of either mutH or mutL or mutS abolishes the growth advantage conferred by the dam mutation to dnaA-deleted cells. Surprisingly, DSBs generated by exposure of the dnaA tus rpo* strain to sub-lethal doses of DNA damaging agents does not allow them to tolerate loss of dnaA. Similarly, SbcCD mediated cleavage of a long palindromic sequence in the lacZ gene was unable to support growth of the dnaA tus rpo* strain.

**Amplification in multiple exo mutant**

Inactivation of at least two exonucleases (xonA and sbcCD or xseA) causes mild amplification of the terminus area and allows dnaA46 tus rpo* xonA sbcCD and dnaA46 tus rpo* xonA xseA strains to grow at the non- permissive temperature (Rudolph et al. 2013, Wendel, Courcelle and Courcelle 2014, Wendel et al. 2018, Midgley-Smith et al. 2019). According to the 3´ flap model (Fig. 3ii) (Midgley-Smith et al. 2019), these exonucleases are required to degrade the 3´ flap generated after fork collision in the terminus, so in their absence the 3´ flap will be used by PriA to restart replication, similarly to what was proposed for the recG mutant. Nevertheless, in contrast to the recG mutant, the amplification seen in these mutants was much weaker despite the suppression of dnaA being efficient (Midgley-Smith et al. 2019). This precludes making a strong link between the extent of amplification in the terminus and suppression of the dnaA requirement for viability but supports the hypothesis of a general upregulation of SDR activity across the genome, terminus amplification being a secondary effect visible due to the fork-trap.

The other model put forward to explain terminus amplification in a multiple exonuclease mutant is the replication fork bypass model. Here, ExoI and SbcCD are proposed to process the intermediate generated by RecC to allow its degradation by RecBCD enzyme complex for completion of replication (Fig. 3iii) (Wendel, Courcelle and Courcelle 2014, Wendel et al. 2018). However, this model does not explain the survival of a dnaA mutant by inactivation of these exonucleases.

**Amplification in rnhA mutant**

As previously noted for oriKs, whole genome sequencing based MFA analysis of the rnhA mutant revealed multiple peaks, including a specific peak in the terminus region (Maduie et al. 2014; Dimude et al. 2015). In contrast to the recG mutant, this peak was very small. Importantly, the rnhA dnaA tus triple mutant showed peaks in the regions flanking terA-terB (and not in between) suggesting that the peak observed in the fork trap region is due to the blockage of forks coming from outside of this region rather than initiated there (Maduie et al. 2014). This gives an informative perspective that a clear peak observed between terA and terB does not necessarily imply phenomena initiated there. Instead, amplification can appear there due to
forks being captured by the fork trap. Despite robust attempts to map specific locations of oriKs these have remained elusive and the question needs further investigation (de Massy, Fayet and Kogoma 1984; Nishitani, Hidaka and Horiuchi 1993; Horiuchi et al. 1994; Kodama et al. 2002; Maduque et al. 2014). Regardless of the positions of oriKs, it is clear that they originate from R-loops and they can support growth of cells upon inactivation of DnaA. This tolerance becomes more effective by addition of MutHLS and they can support growth of cells upon inactivation (Cockram et al. 2019), as this tolerance becomes more effective by addition of MutHLS and they can support growth of cells upon inactivation of DnaA. This tolerance becomes more effective by addition of MutHLS and they can support growth of cells upon inactivation (Cockram et al. 2019).

Random replication fork dependent DSBs (~18%; reviewed in (Michel et al. 2018)) augmented by breaks at Tus/ter blocks could be responsible for SDR initiation in the recG mutant, whereas MutHLS mediated DSBs associated with replication forks are required to support SDR in the dam mutant (Raghunathan et al. 2019). The reverse-restart observed in a recG mutant can explain why random fork breakage can lead to SDR. In addition, in a small proportion of cells where a fork stalls at a Tus/ter barrier in the recG mutant this can lead to a ping-pong amplification of the terminus region via the same reverse-restart reaction. Cells undergoing this ping-pong reaction may be the small subpopulation (~2%) of the recG mutant that are hyper filamented and contain substantially increased numbers of replisomes, when visualized as YPet-DnaA foci (Midgley-Smith et al. 2018). Terminal amplification seen in the recG mutant is higher due to this active ping-pong mechanism, whereas it is lower in rhnA, dam or exo mutants, where the terminus trap may simply block all forks originated by SDR, as proposed previously (Gowrishankar 2015). The event that precipitates SDR in the multiple exo mutant is unknown but this may be where 3′ flaps play a role. It is important to note that the reverse-restart and 3′ flap models are not mutually exclusive, if relevant to different situations. However, as we have discussed earlier, PriA cannot restart replication from a 3′ flap since it needs 3′ end paired to the template to initiate replication. Instead, a 3′ flap (3′ ssDNA) might simply be used for RecA loading and strand invasion and then PriA assembly can restart bidirectional replication.

Moreover, if 3′ flaps are formed under certain mutant conditions, we consider it unlikely that they are generated by the collision of two DNA replication forks, as it has been shown that the replicative helicase DnaB does not unwind a 3′ end when the DNA strand encountered is paired to its template. Instead, DnaB encircles the two strands and moves past the 3′ end while leaving it bound to its template (Kaplan 2000; Kaplan and O’Donnell 2002). This biochemical observation provides a simple mechanism to avoid problems when DNA replication forks meet and it should be noted that any problems, which might be caused by replication fork collisions, are currently hypothetical. In fact, any explanation based on the collision of replication forks cannot satisfactorily explain DNA amplification in a recG mutant, as this is still observed in a linear chromosome where replication forks do not meet (Rudolph et al. 2013; Dimude et al. 2015).

The DNA amplification observed in rhnA and in topA mutants is due to R-loops. It is still not understood what determines the frequency, spatial distribution and stability of the R-loops in the absence of RNaseHI. Nevertheless remarkably, these R-loops are robust enough to support growth of a DnaA inactivated strain even without tus or rpo” alleles.

**DNA degradation of the chromosome terminus region**

Whole genome sequencing based MFA analysis has revealed DNA loss in the chromosome terminus area of a recBC mutant with a maximum loss in the dif/terC region (Rudolph et al. 2013). The first model proposed to explain the DNA loss in a recBC mutant suggested that when replication forks meet, in the terminus region, they tend to by-pass each other and over replicate the DNA (Wendel, Courcelle and Courcelle 2014). The model proposed that RecC facilitates unwinding of this over-replicated

**Conclusions from DNA terminus amplification studies**

Classical and recent studies point to D-loops and R-loops being implicated in SDR and suggest that SDR is intimately associated with terminus DNA amplification. However, the formation of a D-loop is not sufficient to set up SDR since most D-loops are expected to be resolved to restore normal DNA replication (e.g. one-ended DSB repair of a broken replication fork) or an intact chromosome (e.g. two ended DSB repair at the site of ionizing radiation or similar damage) (Fig. 1). What is needed for SDR initiated at a D-loop is the establishment of a replication fork proceeding around the chromosome in the reverse direction to a normal fork. This implies some sort of abnormal reaction. One such reaction is the over-replication of a section of DNA prior to D-loop formation. This is the case for the 3′ flap model (Rudolph, Upton and Lloyd 2009; Rudolph et al. 2013) and the reverse-restart model (Azeroglu et al. 2016). Both of these models propose that the initial over-replication event occurs prior to D-loop formation and that the D-loop creates a new replication bubble with forks that can proceed in both directions round the chromosome. The lack of an abnormal reaction leading to a reverse orientated fork is presumably the reason why DSBs generated by DNA damaging agents do not support the growth of a ∆dnaA tus rpoB” strain (Raghunathan et al. 2019). SbcCD dependent cleavage of a long DNA palindrom in the lacZ gene was shown to generate a two-ended DSB (Eykenboom et al. 2008) that can be processed in 50% of cases to a broken replication fork (Cockram et al. 2015). Since both of these structures are repaired without setting up reversely oriented replication forks, palindrome cleavage is also unable to rescue the absence of DnaA in ∆dnaA tus rpoB” strain (Eykenboom et al. 2008; Raghunathan et al. 2019). Presumably D-loops set up in recG, dam and multiple exo mutants have the characteristic of enabling replication forks to be established in the reverse chromosomal direction allowing the strains to tolerate the absence of dnaA, provided two major obstacles of replication-transcription collision and Tus/ter blockage are removed by rpo” and tus mutations respectively.
DNA loss was observed in the terminus under the growth conditions where terminus performed to check if any DSBs could be detected in the chromosome (Dimude, Midgley-Smith and Rudolph 2018). The observation made it clear that the terminus-localized DNA localized DSBs (or DSEs) are formed in wild type cells. Instead, RecA binding was also seen in a recBC mutant derivative of a strain harboring an ectopic site that generates a new fork trap close to terA rather than at dif/terC. DNA loss focused at dif/terC was also seen in a recBC mutant derivative of a strain harboring an ectopic site, because only in the absence of replication fork repair by RecBCD (Fig. 3iii) (Wendel, Courcelle and Courcelle 2014; Courcelle et al. 2018). In this second model (Fig. 4), randomly located foci persist (Fig. 4cde). This then causes a problem when the cell tries to segregate its replicated chromosomes and divide (Fig. 4f). The terminus region of one of the chromosomes (the linear tail of the sigma structure) becomes trapped as the septum attempts to close and this attempted cell division leads to chromosome breakage (Fig. 4f). The initial break of the replication fork is randomly located on the chromosome and so cannot be detected in a population of cells (Fig. 4c). It is independent of recBC but persists in a recBC mutant. The subsequent break occurs in the dif/terC region but only if the cell is a recBC mutant, because only in the absence of replication fork repair by RecBCD does the sigma-replicating chromosome persist and get trapped in the closing septum at cell division (Fig. 4e-f). This subsequent break in the dif/terC region generates one cell with a linear chromosome, which is doomed to die, and a second cell with a circular chromosome containing a short linear tail in the terminus (Fig. 4g). If this mark persists until the arrival of the next round of replication forks, a new sigma-shaped chromosome is generated and the cleavage cycle is repeated causing the heritability of terminus breakage that has been documented (Fig. 4g-j) (Sinha et al. 2017, 2018).

This model has been confirmed by many tests. MFA by genomic sequencing has shown that terminus DNA loss in a recBC mutant is not dependent on the action of dif/XerC but is dependent on cell division and is focused in the dif/terC region by the action of FtsK (Sinha et al. 2017). Fluorescence microscopy, in which yGFP-ParS<sub>SMRT2</sub> binds to a parS<sub>SMRT2</sub> site inserted between dif and terC, has been used to analyze terminus DNA loss in the recBC mutant at a single cell level (Sinha et al. 2017, 2018). In recBC mutant cells, the terminus is normally replicated and two foci are clearly visible after replication. In 18% of cell divisions, one of these two foci is lost and one daughter cell is born without a focus and does not grow further. The other cell, which keeps a focus, grows and again replicates the parS<sub>SMRT2</sub> site so that two foci become visible. However, one focus is again lost during division making this DNA loss heritable. The cell with the linear chromosome that loses its focus does not grow because of the action of the HipA toxin, which is more stable than the HipB antitoxin encoded by the hipBA operon that is located in the terminus and is degraded rapidly following DSB formation (Sinha et al. 2018). More than two terminus foci were never observed, despite cell division-mediated focus-loss being observed much later than replication was complete, contrary to a prediction of amplification preceding DNA loss, as proposed in the first model. After the cell division induced breakage of the chromosomes in the recB mutant, the DSEs generated at the terminus are degraded by exonucleases and SbcCD plays major role in that end degradation (Dimude, Midgley-Smith and Rudolph 2018). Further evidence that collisions of replication forks are not implicated in terminus DNA loss was obtained by investigating the phenomenon in a phage N15 lysogen where the TelN gene product processes a tos site to generate a linear chromosome with two hairpin capped ends. In cells with this linear chromosome, recBC dependent terminus DNA loss was still observed despite converging replication forks not meeting in the terminus (Dimude, Midgley-Smith and Rudolph 2018; Sinha et al. 2018). The pattern of degradation was affected by the presence or absence of the HipA HipB toxin-antitoxin system with the cell inheriting a broken chromosome containing the hipBA operon being poisoned by the HipA toxin and showing little degradation (Sinha et al. 2017; Dimude, Midgley-Smith and Rudolph 2018). Moving the site of linearization 200 kb away from the hipBA operon and dif/terC allowed more symmetrical degradation and a displaced location of maximum degradation, consistent with a delay to the degradation of hipBA and a preferred location of cleavage that is determined by the DNA lying in the path of the septum when the cell tries to divide, rather than the presence of a specific site of cleavage (Dimude, Midgley-Smith and Rudolph 2018).

Conclusions from the terminus degradation studies

Studies of terminus DNA loss observed in a recBC mutant have revealed a new and unexpected phenomenon, the cleavage of a sigma-replicating chromosome at cell division (Fig. 4e-j) (Sinha et al. 2018). The mechanism of this cleavage is still unknown and may or may not be related to the guillotining of chromosome.
Figure 4. Processing of a broken replication fork in wild type (wt), ΔrecA and ΔrecBCD cells. DNA replication initiates at a fixed position known as oriC (denoted by a small circle) on a circular chromosome (a) and progresses bidirectionally (b). Encounter of the replication fork with a nick on the template strand (c) leading to a broken replication fork (replication fork collapse) and generation of a DSE (one-ended DSB) at the fork (depicted by 1 in the figure). This can lead to cell division-dependent DNA cleavage of the terminus in recBCD mutant cells (depicted by 2 in the figure). In wt cells, the broken fork is repaired by RecBCD-RecA mediated recombinational repair, to re-establish an intact replication fork (from c to b). In ΔrecA cells, RecBCD binds to this DSE and degrades the entire broken arm, to generate an intact single circular chromosome (from d to a). However, the degradation process of the entire broken arm by RecBCD needs assistance from exonucleases (such as Exol, SbcCD etc.), since frequent encounter with Chi sequences by RecBCD leads to the generation of long 3′-ssDNA overhangs and disassociation of RecBCD. Owing to its ability to bind only blunt (or nearly blunt) DSEs, RecBCD is unable to bind and restart degradation from these long 3′ ssDNA overhangs. ssDNA exonucleases degrade the overhangs to make them blunt and so facilitate RecBCD loading and further degradation. In ΔrecBCD cells, the broken arm persists. The other intact replication fork continues to progress towards the terminus (e) and reaches an appropriately oriented ter site (shown here as red line). Since replication and segregation occur simultaneously in bacterial cells, the intact circular chromosome and the broken linear chromosome segregate into two halves of the cell but remain linked by the intact fork in the terminus (e). FtsK action aligns two dif sites (shown here as green lines), one on the circular and another on the linear chromosome, in the middle of the septum. Septum formation causes guillotining of the chromosome in the dif region of the terminus, separating linear and circular chromosomes into two daughter cells (f). The cell inheriting the linear chromosome does not grow further. The cell inheriting the circular chromosome has a replication fork attached to a short linear DNA tail that remains in the terminus (g). A new round of replication initiated at oriC sends forks around the chromosome (h), which merge with the previous fork in the terminus, causing the short linear tail to be enlarged to an entire broken chromosome arm (i). Attempted segregation followed by guillotining (j) makes the process heritable (from g to j and then back to g).
dimers in dif or xerCD mutants (Hendricks et al. 2000; Prikryl, Hendricks and Kuempel 2001). The work by Sinha et al. (2018) has also provided an estimate for the frequency of replication fork breakage of 18% of replication cycles in cells growing in a minimal glucose medium at 30 °C. The heritability of the phenomenon explains the overall poor viability of the recBC mutant. The tests of the mechanism show that the loss of DNA in the terminus occurs because of breaks that occur during cell division only in recBC mutant cells, but the initiating event is the formation of a sigma-replicating chromosome by lack of repair of a broken DNA replication fork. In the recBC mutants, the terminus DNA ends, generated by cleavage, are degraded by other nucleases, with an important role for SbcCD (Fig. 4) (Dimude, Midgley-Smith and Rudolph 2018).

**GENERAL CONCLUSIONS**

Several studies in the last decade have provided vital novel insights and have given different perspectives on the roles of DNA replication and DSB repair proteins in maintaining bacterial chromosome integrity and stable DNA replication (SDR). Thanks primarily to studies of the bacterium *E. coli*, now we know that SDR induced in many of these mutants (not only in *rnhA*, where it was originally characterized) has the potential to replicate the entire chromosome and to permit survival without oriC mediated replication initiation. Remarkably, chromosome replication by SDR has only two obstacles to overcome: 1. to cross fork trap barriers and, 2. to survive the consequences of head-on conflicts with the transcriptional complex. Amazingly, *E. coli* chromosome organization and replication-transcription orientation have evolved in a way that efficiently uses these two restrictions to ensure that any chromosomal region except the terminus is replicated in only one direction. Recombination-dependent, origin-independent DNA replication has not yet been observed for other bacterial species. However, our increased understanding of the *E. coli* system opens up the possibility of making such observations using appropriate mutants. It is a well-known mechanism of DNA replication in the late lytic cycle of bacteriophage T4 and could permit the survival of the archaeon *H. volcanii* lacking its four origins of DNA replication. It may indeed represent a primitive mechanism of DNA replication initiation.

*E. coli* terminus DNA amplification has been proposed to be associated with aberrant events, hypothesized to occur when DNA replication forks collide, in the absence of key proteins that normally remove hypothetical aberrant DNA structures. However, we question whether the merging of DNA replication forks actually leads to such aberrant events in living cells. Further studies are required to clarify the mechanisms responsible for terminus DNA amplification in *E. coli* and to determine whether similar events can be observed in other bacterial species. Terminus DNA loss in an *E. coli* recBC mutant has revealed the existence of a new mechanism for the generation of DSBs, which involves the cleavage of DNA trapped at the septum during cell division. The details of this cleavage mechanism are yet to be understood, its phylogenetic distribution revealed, and its evolutionary value determined.

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**REFERENCES**

Al-Deib AA, Mahdi AA, Lloyd RG. Modulation of recombination and DNA repair by the RecG and PriA helicases of *Escherichia coli* K-12. *J Bacteriol* 1996;178:6782–9.

Anderson DG, Kowalczykowski SC. The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a chi-regulated manner. *Cell* 1997;90:77–86.

Asai T, Bates DB, Kogoma T. DNA replication triggered by double-stranded breaks in *E. coli*: dependence on homologous recombination functions. *Cell* 1994;78:1051–61.

Asai T, Imai M, Kogoma T. DNA damage-inducible replication of the *Escherichia coli* chromosome is initiated at separable sites within the minimal oric. *J Mol Biol* 1994;235:1459–69.

Asai T, Kogoma T. D-loops and R-loops: alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. *J Bacteriol* 1994a;176:1807–12.

Asai T, Kogoma T. Roles of *ruvA*, *ruvC* and recG gene functions in normal and DNA damage-inducible replication of the *Escherichia coli* chromosome. Genetics 1994b;137:895–902.

Asai T, Kogoma T. The RecF pathway of homologous recombination can mediate the initiation of DNA damage-inducible replication of the *Escherichia coli* chromosome. *J Bacteriol* 1994c;176:7113–4.

Asai T, Sommer S, Bailone A et al. Homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in *Escherichia coli*. EMBO J 1993;12:3287–95.

Ausiannikava D, Allers T. Diversity of DNA replication in the archaea. Genes 2017;8:56.

Ayora S, Carrasco B, Cardenas PP et al. Double-strand break repair in bacteria: a view from *Bacillus subtilis*. FEMS Microbiol Rev 2011;35:1055–81.

Azeroglu B, Mawer JS, Cockram CA et al. RecG directs DNA synthesis during double-strand break repair. PLoS Genetics 2016;12:e1005799.

Benthchikou E, Servant P, Coste G et al. A major role of the RecFOR pathway in DNA double-strand-break repair through ESDSA in *Deinococcus radiodurans*. PLoS Genetics 2010;6:e1000774.

Bhattacharyya B, George NP, Thurmes TM et al. Structural mechanisms of PriA-mediated DNA replication restart. Proc Natl Acad Sci USA 2014;111:1373–8.

Biek DP, Cohen SN. Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. *J Bacteriol* 1986;167:594–603.

Brochu J, Vlachos-Breton E, Sutherland S et al. Topoisomerases I and III inhibit R-loop formation to prevent unregulated replication in the chromosomal Ter region of *Escherichia coli*. PLoS Genetics 2018;14:e1007668.

Capaldo FN, Barbour SD. DNA content, synthesis and integrity in dividing and non-dividing cells of rec strains of *Escherichia coli* K12. *J Mol Biol* 1975a;91:53–66.

Capaldo FN, Barbour SD. The role of the rec genes in the viability of *Escherichia coli* K12. Basic Life Sci 1975b;5a:405–18.

Capaldo FN, Ramsey G, Barbour SD. Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12. *J Bacteriol* 1974;118:242–9.
Clark AJ, Margulies AD. Isolation and characterization of recombination-deficient mutants of Escherichia coli K12. Proc Natl Acad Sci USA 1965;52:451–9.

Cockram CA, Filatenkova M, Danos V et al. Quantitative genomic analysis of RecA protein binding during DNA double-strand break repair reveals RecBCD action in vivo. Proc Natl Acad Sci USA 2015;112:E4735–4742.

Cohen A, Clark AJ. Synthesis of linear plasmid multimers in Escherichia coli K-12. J Bacteriol 1986;167:327–35.

Connelly JC, de Leau ES, Leach DR. DNA cleavage and degradation by the SbcCD protein complex from Escherichia coli. Nucleic Acids Res 1999;27:1039–46.

Connelly JC, Kirkham LA, Leach DR. The SbcCD nuclease of Escherichia coli is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. Proc Natl Acad Sci USA 1998;95:7969–74.

Costes A, Lecointe F, McGovern S et al. The C-terminal domain of the bacterial SSB protein acts as a DNA maintenance hub at active chromosome replication forks. PLoS Genetics 2010;6:e1001238.

Courcelle J, Wendel BM, Livingstone DD et al. RecBCD is required to complete chromosomal replication: Implications for double-strand break frequencies and repair mechanisms. DNA Repair 2015;32:86–95.

Dabert P, Ehrlich SD, Gruss A. Chi sequence protects against RecBCD degradation of DNA in vivo. Proc Natl Acad Sci USA 1992;89:12073–7.

Dabert P, Ehrlich SD, Gruss A. High-molecular-weight linear multimer formation by single-stranded DNA plasmids in Escherichia coli. J Bacteriol 1992;174:173–8.

Daly MJ, Gaidamakova EK, Matrosova VV et al. Accumulation of Mn(II) in Deinococcus radiodurans facilitates gamma-radiation resistance. Science (New York, NY) 2004;306:1025–8.

de Massy B, Fayet O, Kogoma T. Multiple origin usage for DNA replication in sdrA(rnh) mutants of Escherichia coli K-12. Initiation in the absence of oriC. J Mol Biol 1984;178:227–36.

Dillingham MS, Kowalczykowski SC. RecBCD enzyme and the repair of double-stranded DNA breaks. Microbiol Mol Biol Rev 2008;72:642–71, Table of Contents.

Dimude JU, Midgley-Smith SL, Rudolph CJ. Replication-transcription conflicts trigger extensive DNA degradation in Escherichia coli cells lacking RecBCD. DNA Repair 2018;70:37–48.

Dimude JU, Midgley-Smith SL, Stein M et al. Replication termination: containing fork fusion-Mediated pathologies in Escherichia coli. Genes 2016;7:40.

Dimude JU, Stockum A, Midgley-Smith SL et al. The consequences of replicating in the wrong orientation: bacterial chromosome duplication without an active replication origin. mBio 2015;6:e01294–01215.

Drolet M, Brochu J. R-loop-dependent replication and genomic instability in bacteria. DNA Repair 2019;84:102693.

Eykelenboom JK, Blackwood JK, Okely E et al. SbcCD causes a double-strand break at a DNA palindrome in the Escherichia coli chromosome. Mol Cell 2008;29:644–51.

Fujiwara Y, Tatsumi M. Replicative bypass repair of ultraviolet damage to DNA of mammalian cells: caffeine sensitive and caffeine resistant mechanisms. Mutation Res 1976;37:91–110.

Gowrishankar J. End of the beginning: elongation and termination features of alternative modes of chromosomal replication initiation in bacteria. PLoS Genetics 2015;11:e1004909.

Hamilton NA, Wendel BM, Weber EA et al. RecBCD, SbcCD and ExoI process a substrate created by convergent replisomes to complete DNA replication. Mol Microbiol 2019;111:1638–51.

Handa N, Yang L, Dillingham MS et al. Molecular determinants responsible for recognition of the single-stranded DNA regulatory sequence, chi, by RecBCD enzyme. Proc Natl Acad Sci USA 2012;109:8901–6.

Heller RC, Marians KJ. Unwinding of the nascent lagging strand by Rep and PriA enables the direct restart of stalled replication forks. J Biol Chem 2005;280:34143–51.

Hendricks EC, Szerlong H, Hill T et al. Cell division, goulitoning of dimer chromosomes and SOS induction in resolution mutants (dij, xerC and xerD) of Escherichia coli. Mol Microbiol 2000;36:973–81.

Higgins NP, Kato K, Strauss B. A model for replication repair in mammalian cells. J Mol Biol 1976;101:417–25.

Hong X, Cadwell GW, Kogoma T. Escherichia coli RecG and RecA proteins in R-loop formation. EMBO J 1995;14:2385–92.

Horii Z, Clark AJ. Genetic analysis of the recF pathway to genetic recombination in Escherichia coli K12: isolation and characterization of mutants. J Mol Biol 1973;80:327–44.

Horiiuchi T, Fujimura Y, Nishitani H et al. The DNA replication fork blocked at the Ter site may be an entrance for the RecBCD enzyme into duplex DNA. J Bacteriol 1994;176:4656–63.

Ireton K, Grossman AD. Coupling between gene expression and DNA synthesis early during development in Bacillus subtilis. Proc Natl Acad Sci USA 1992;89:8808–12.

Ivanova D, Taylor T, Smith SL et al. Shaping the landscape of the Escherichia coli chromosome: replication-transcription encounters in cells with an ectopic replication origin. Nucleic Acids Res 2015;43:7865–77.

Jaktaji RP, Lloyd RG. PriA supports two distinct pathways for replication restart in UV-irradiated Escherichia coli cells. Mol Microbiol 2003;47:1091–100.

Kalman M, Murphy H, Cashel M. The nucleotide sequence of recG, the distal spo operon gene in Escherichia coli K-12. Gene 1992;110:95–99.

Kaplan DL, O’Donnell M. DnaB drives DNA branch migration and dislodges proteins while encircling two DNA strands. Mol Cell 2002;10:647–57.

Kaplan DL. The 3’-tail of a forked-duplex sterically determines whether one or two DNA strands pass through the central channel of a replication-fork helicase. J Mol Biol 2000;301:285–99.

Khan SR, Kuzminov A. Replication forks stalled at ultraviolet lesions are rescued via RecA and RuvABC protein-catalyzed disintegration in Escherichia coli. J Biol Chem 2012;287:6250–65.

Khan SR, Kuzminov A. Thymineless death in Escherichia coli is unaffected by chromosomal replication complexity. J Bacteriol 2019;201:e00797–18.

Khan SR, Mahaseth T, Kouzminova EA et al. Static and dynamic factors limit chromosomal replication in Escherichia coli, avoiding dangers of runaway overreplication. Genetics 2016;202:945–60.

Kodama K, Kobayashi T, Niki H et al. Amplification of Hot DNA segments in Escherichia coli. Mol Microbiol 2002;45:1575–88.

Kogoma T, Cadwell GW, Barnard KG et al. The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. J Bacteriol 1996;178:1258–64.

Kogoma T, Lark KG. Characterization of the replication of Escherichia coli DNA in the absence of protein synthesis: stable DNA replication. J Mol Biol 1975;94:243–56.

Kogoma T, Lark KG. DNA replication in Escherichia coli: replication in absence of protein synthesis after replication inhibition. J Mol Biol 1970;52:143–64.
Kogoma T, Skarstad K, Boye E et al. RecA protein acts at the initiation of stable DNA replication in msr mutants of Escherichia coli K-12. J Bacteriol 1985;163:439–44.

Kogoma T, Torrey TA, Connaughton MJ. Induction of UV-resistant DNA replication in Escherichia coli: induced stable DNA replication as an SOS function. Mol Gen Gene: MGG 1979;176:1–9.

Kogoma T, von Meyenburg K. The origin of replication, oriC, and the dnaA protein are dispensable in stable DNA replication (sdrA) mutants of Escherichia coli K-12. EMBO J 1983;2:463–8.

Kogoma T. A novel Escherichia coli mutant capable of DNA replication in the absence of protein synthesis. J Mol Biol 1978;121:55–69.

Kogoma T. RNAse H-defective mutants of Escherichia coli. J Bacteriol 1986;166:361–3.

Kogoma T. Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. Microbiol Mol Biol Rev 1997;61:212–38.

Kouzminova EA, Kadyrov FF, Kuzminov A. RNAse HII saves msrA mutant Escherichia coli from R-loop-associated chromosomal fragmentation. J Mol Biol 2017;429:2873–94.

Kreuzer KN, Brister JR. Initiation of bacteriophage T4 DNA replication and replication fork dynamics: a review in the Virolology Journal series on bacteriophage T4 and its relatives. Virol J 2010;7:358.

Kreuzer KN, Saunders M, Weislo LJ et al. Recombination-dependent DNA replication stimulated by double-strand breaks in bacteriophage T4. J Bacteriol 1995;177:684–53.

Kreuzer KN, Yap WY, Menkens AE et al. Recombination-dependent replication of plasmids during bacteriophage T4 infection. J Biol Chem 1988;263:11366–73.

Kuong KJ, Kuzminov A. Disintegration of nascent replication bubbles during thymine starvation triggers RecA- and RecBCD-dependent replication origin destruction. J Biol Chem 2012;287:23958–70.

Kusano K, Nakayama K, Nakayama H. Plasmid-mediated lethal and plasmid multimer formation in an Escherichia coli recBC sbcBC mutant. Involvement of RecF recombination pathway genes. J Mol Biol 1989;209:623–34.

Kushner SR, Nagaishi H, Clark AJ. Indirect suppression of recB and recC mutations by exonuclease I deficiency. Proc Natl Acad Sci USA 1972;69:1366–70.

Kushner SR, Nagaishi H, Templin A et al. Genetic recombination in Escherichia coli: the role of exonuclease I. Proc Natl Acad Sci USA 1971;68:824–7.

Kuzminov A, Schabtach E, Stahl FW. Chi sites in combination with RecA protein increase the survival of linear DNA in Escherichia coli by inactivating exoV activity of RecBCD nuclease. EMBO J 1994;13:2764–76.

Kuzminov A, Stahl FW. Stability of linear DNA in recA mutant Escherichia coli cells reflects ongoing chromosomal DNA degradation. J Bacteriol 1997;179:880–8.

Kuzminov A. Collapse and repair of replication forks in Escherichia coli. Mol Microbiol 1995;16:373–84.

Kuzminov A. Homologous recombination-experimental systems, analysis, and significance. EcoSal Plus 2011:4.

Kuzminov A. Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda. Microbiol Mol Biol Rev 1999;63:751–813.

Kuzminov A. Single-strand interruptions in replicating chromosomes cause double-strand breaks. Proc Natl Acad Sci USA 2001;98:8241–6.

Lark KG, Lark CA. recA-dependent DNA replication in the absence of protein synthesis: characteristics of a dominant lethal replication mutation, dnaT, and requirement for recA+ function. Cold Spring Harbor Sympos Quantitative Biol 1979;43 Pt 1:537–49.

Leach DR, Okely EA, Pinder DJ. Repair by recombination of DNA containing a palindromic sequence. Mol Microbiol 1997;26:597–606.

Lecomte F, Serena C, Velten M et al. Anticipating chromosomal replication fork arrest: SSB targets repair DNA helicases to active forks. EMBO J 2007;26:4239–51.

Lee EH, Kornberg A. Replication deficiencies in priA mutants of Escherichia coli lacking the primosomal replication n’ protein. Proc Natl Acad Sci USA 1991;88:3029–32.

Lenhart JS, Schroeder JW, Walah BW et al. DNA repair and genome maintenance in Bacillus subtilis. Microbiol Mol Biol Rev 2012;76:530–64.

Lisby M, Mortensen UH, Rothstein R. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nature Cell Biol 2003;5:572–7.

Liu J, Morrical SW. Assembly and dynamics of the bacteriophage T4 homologous recombination machinery. Virol J 2010;7:357.

Lloyd RG, Benson FE, Shurvinton CE. Effect of ruv mutations on recombination and DNA repair in Escherichia coli K12. Molecular & Gen Gen: MGG 1984;194:303–9.

Lloyd RG, Buckman C. Genetic analysis of the recG locus of Escherichia coli K-12 and of its role in recombination and DNA repair. J Bacteriol 1991;173:1004–11.

Lloyd RG, Buckman C. Identification and genetic analysis of sbcC mutations in commonly used recBC sbcB strains of Escherichia coli K-12. J Bacteriol 1985;164:836–44.

Lloyd RG, Pcksley SM, Prescott C. Inducible expression of a gene specific to the RecF pathway for recombination in Escherichia coli K12. Mol Gen Gene: MGG 1983;190:162–7.

Lloyd RG, Sharples GJ. Dissociation of synthetic Holliday junctions by E. coli RecG protein. EMBO J 1993;12:17–22.

Lloyd RG. Conjugational recombination in resolvase-deficient ruvC mutants of Escherichia coli K-12 depends on recG. J Bacteriol 1991;173:5414–8.

Lovett ST, Clark AJ. Genetic analysis of regulation of the RecF pathway of recombination in Escherichia coli K-12. J Bacteriol 1983;153:1471–8.

Lovett ST, Luisi-Deluca C, Kolodner RD. The genetic dependence of recombination in recD mutants of Escherichia coli. Genetics 1988;120:37–45.

Luder A, Mosig G. Two alternative mechanisms for initiation of DNA replication forks in bacteriophage T4: priming by RNA polymerase and by recombination. Proc Natl Acad Sci USA 1982;79:1101–5.

Maduikene NZ, Taherani AK, Wang JD et al. Replication of the Escherichia coli chromosome in RNase HI-deficient cells: multiple initiation regions and fork dynamics. Mol Microbiol 2014;91:39–56.

Maggi TR, Asai T, Malka D et al. DNA damage-inducible origins of DNA replication in Escherichia coli. EMBO J 1992;11:4219–25.

Maggi TR, Kogoma T. Requirement of RecBC enzyme and an elevated level of activated RecA for induced stable DNA replication in Escherichia coli. J Bacteriol 1990;172:1833–49.

Martel M, Balleydier A, Sauriol A et al. Constitutive stable DNA replication in Escherichia coli cells lacking type 1A topoisomerase activity. DNA repair 2015;35:37–47.

Masse E, Drolet M. Escherichia coli DNA topoisomerase I inhibits R-loop formation by relaxing transcription-induced negative supercoiling. J Biol Chem 1999;274:16659–64.
McGlynn P, Lloyd RG. Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell 2000;101:35–45.

Michel B, Leach D. Homologous recombination-enzymes and pathways. EcoSal Plus 2012;1:58.

Michel B, Sandler SJ. Replication restart in bacteria. J Bacteriol 2017;199:e00102–17.

Michel B, Sinha AK, Leach DRF. Replication fork breakdown and restart in Escherichia coli. Microbiol Mol Biol Rev 2018;82:e00013–18.

Midgley-Smith SL, Dimude JU, Rudolph CJ. A role for 3′ exonucleases at the final stages of chromosome duplication in Escherichia coli. Nucleic Acids Res 2019;47:1847–60.

Midgley-Smith SL, Dimude JU, Taylor T et al. Chromosomal over-replication in Escherichia coli recG cells is triggered by replication fork fusion and amplified if replicore symmetry is disturbed. Nucleic Acids Res 2018;46:7701–15.

Million-Weaver S, Samadpour AN, Merrikh H. Replication restart from replication fork collision fork progression.

Miranda A, Kuzminov A. Chromosomal lesion suppression and removal in Escherichia coli via linear DNA degradation. Genetics 2003;163:1255–71.

Nassif N, Penney J, Pal S et al. Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. Mol Cell Biol 1994;14:1613–25.

Niki H, Ichinose C, Ogura T et al. Chromosomal genes essential for stable maintenance of the mini-F plasmid in Escherichia coli. J Bacteriol 1995;179:2374–82.

Niki H, Ogura T, Hiraga S. Linear multimer formation of plasmid DNA in Escherichia coli hopE (recD) mutants. Mol Gen Gene: MGG 1990;224:1–9.

Nishitani H, Hidaka M, Horiiuchi T. Specific chromosomal sites enhancing homologous recombination in Escherichia coli mutants defective in RNase H. Mol Gene Gene: MGG 1993;240:307–14.

Nurse P, Zavitz KH, Marians KJ. Inactivation of the Escherichia coli PriA DNA replication protein induces the SOS response. J Bacteriol 1991;173:6686–93.

Nussbaum A, Cohen A. Use of a bioluminescence gene reporter for the investigation of red-dependent and gam-dependent plasmid recombination in Escherichia coli K12. J Mol Biol 1988;203:391–402.

Prikryl J, Hendricks EC, Kuempel PL. DNA degradation in the terminus region of resolvase mutants of Escherichia coli, and suppression of this degradation and the Dif phenotype by recD. Biochimie 2001;83:171–6.

Raghunathan N, Goswami S, Leela JK et al. A new role for Escherichia coli Dam DNA methylase in prevention of aberrant chromosomal replication. Nucl Acids Res 2019;47:5698–711.

Rudolph CJ, Upton AL, Harris L et al. Pathological replication in cells lacking RecG DNA translocase. Mol Microbiol 2009;73:352–66.

Rudolph CJ, Upton AL, Lloyd RG. Replication fork collisions cause pathological chromosomal amplification in cells lacking RecG DNA translocase. Mol Microbiol 2009;74:940–55.

Rudolph CJ, Upton AL, Stockum A et al. Avoiding chromosome pathology when replication forks collide. Nature 2013;500:608–11.

Sanchez H, Kidane D, Castillo Cozar M et al. Recruitment of Bacillus subtilis RecN to DNA double-strand breaks in the absence of DNA end processing. J Bacteriol 2006;188:353–60.

Sandler SJ, Samra HS, Clark AJ. Differential suppression of priA2- kan phenotypes in Escherichia coli K-12 by mutations in priA, lexA, and dnaC. Genetics 1996;143:5–13.

Schapiro IM, Libby SJ, Fang FC. Inhibition of bacterial DNA replication by zinc mobilization during nitrosative stress. Proc Natl Acad Sci USA 2003;100:8496–501.

Seigmeur M, Ehrlich SD, Michel B. recB sbcB sbcD mutants are deficient in recombinational repair of UV lesions by RecBC. J Bacteriol 1999;181:6220–1.

Silverstein Z, Cohen A. Synthesis of linear multimers of OriC and pBR322 derivatives in Escherichia coli K-12: role of recombination and replication functions. J Bacteriol 1987;169:3131–7.

Sinha AK, Durand A, Desfontaines JM et al. Division-induced DNA double strand breaks in the chromosome terminus region of Escherichia coli lacking RecBCD DNA repair enzyme. PLoS genetics 2013;13:e1006895.

Sinha AK, Pavankumar TL, Kamisetty S et al. Replication arrest is a major threat to growth at low temperature in Antarctic Pseudomonas syringae Lz4W. Mol Microbiol 2013;89:792–810.

Sinha AK, Possoz C, Durand A et al. Broken replication forks trigger heritable DNA breaks in the terminus of a circular chromosome. PLoS genetics 2018;14:e1007256.

Skalka A. A replicator’s view of recombination (and repair). In: Grell RF (eds). Mechanisms in Recombination. Boston, MA: Springer, 1974.

Skarstad K, Boye E. Degradation of individual chromosomes in recA mutants of Escherichia coli. J Bacteriol 1993;175:5505–9.

Smith GR. How RecBCD enzyme and Chi promote DNA break repair and recombination: a molecular biologist’s view. Microbiol Mol Biol Rev 2012;76:217–28.

Storm PK, Hoeckstra WP, de Haan PG et al. Genetic recombination in Escherichia coli. IV. Isolation and characterization of recombination-deficiency mutants of Escherichia coli K12. Mutation Res 1971;13:9–17.

Tanaka T, Masai H. Stabilization of a stalled replication fork by concerted actions of two helicases. J Biol Chem 2006;281:3484–93.

Taylor AF, Amundsen SK, Guttman M et al. Control of RecBCD enzyme activity by DNA binding- and Chi hotspot-dependent conformational changes. J Mol Biol 2014;426:3479–99.

Templin A, Kushner SR, Clark AJ. Genetic analysis of mutations indirectly suppressing recB and recC mutations. Genetics 1972;72:105–15.

Thoms B, Borchers I, Wackernagel W. Effects of single-strand DNases ExoI, RecJ, ExoVII, and SbcCD on homologous recombination of recBC’ strains of Escherichia coli and roles of SbcB15 and XonA2 ExoI mutant enzymes. J Bacteriol 2008;190:179–92.

Thoms B, Wackernagel W. Interaction of RecBCD enzyme with DNA at double-strand breaks produced in UV-irradiated Escherichia coli: requirement for DNA end processing. J Bacteriol 1998;180:5639–45.

Wendel BM, Cole JM, Courcelle CT et al. SbcC-SbcD and ExoI process convergent forks to complete chromosome replication. Proc Natl Acad Sci USA 2018;115:349–54.

Wendel BM, Courcelle CT, Courcelle J. Completion of DNA replication in Escherichia coli. Proc Natl Acad Sci USA 2014;111:16454–9.

White MA, Azeroglu B, Lopez-Vernaza MA et al. RecBCD coordinates repair of two ends at a DNA double-strand break, preventing aberrant chromosome amplification. Nucleic Acids Res 2018;46:6670–82.
White MA, Darmon E, Lopez-Vernaza MA et al. DNA double strand break repair in Escherichia coli perturbs cell division and chromosome dynamics. PLoS Genetics 2020;16:e1008473.

Zahradka K, Buljubasic M, Petranovic M et al. Roles of ExoI and SbcCD nucleases in “reckless” DNA degradation in recA mutants of Escherichia coli. J Bacteriol 2009;191:1677–87.

Zahradka K, Simic S, Buljubasic M et al. sbcB15 and ΔsbcB mutations activate two types of RecF recombination pathways in Escherichia coli. J Bacteriol 2006a;188:7562–71.

Zahradka K, Slade D, Bailone A et al. Reassembly of shattered chromosomes in Deinococcus radiodurans. Nature 2006b;443:569–73.