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Single-strand gap repair involves both RecF and RecBCD pathways

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Abstract Homologous recombination repairs discontinuities in DNA including single-strand gaps (SSGs) and double-strand breaks (DSBs). This commentary describes how the RecBCD and RecF pathways might be exchangeable for the repair of their respective DSB and SSG canonical substrates. In particular, I will discuss how the RecBCD pathway could engage in the repair of an SSG even when the latter is not associated with a DSB.

Keywords DNA repair · Homologous recombination · Broken chromosomes · DNA replication · RecA · RecBCD · RecF · Double-strand break · Single-strand gap · DNA damage

Homologous recombination (HR) is an essential mechanism involved in DNA damage repair. In the bacterium *Escherichia coli*, RecA protein polymerizes around single-stranded DNA (ssDNA) to form a nucleoprotein filament that will invade a homologous duplex DNA, allowing the exchange of DNA strands (for a review, see Kuzminov 1999).

Two types of DNA damages are substrates for HR: (1) single-strand gaps (SSGs, also named daughter-strand

gaps) arise during replication, when DNA synthesis is blocked by a lesion on one of the strands; and (2) double-strand breaks (DSBs) which are induced by ionizing radiation, or can arise during replication through an unrepaired SSG.

While RecA is the key protein involved in the repair of these damages by homologous recombination, two distinct recombination pathways have been originally described (Clark 1973): the RecF and the RecBCD pathways. Both are involved in the pre-synapsis stage of HR, which consists in assembling the nucleofilament of RecA onto ssDNA. The RecF pathway is involved in SSG repair where it mediates the loading RecA protein onto SSB-coated ssDNA that is already present at the gap (Morimatsu and Kowalczykowski 2003). Since no extensive ssDNA is initially present at a DSB, the RecBCD helicase/nuclease complex processes the ends by resection and concomitantly loads RecA on the generated ssDNA overhangs (Anderson and Kowalczykowski 1997, for a review, see Dillingham and Kowalczykowski 2008). The following steps of HR are common to both pathways: search for homology and exchange of DNA strands occur during the synapsis stage. Branch migration and resolution of the Holliday junction completes the HR process leading to the recombinant molecule (Kuzminov 1999; Spies and Kowalczykowski 2005).

The conventional view of two separate specialized pathways for SSG and DSB repair has changed after several studies reported that RecF is also involved in DSB repair. In normal situations, RecBCD accounts for about 99 % of DSB repair, as measured by conjugational recombination. However, a combined inactivation of RecBCD and exonuclease I results in repair of DSBs by RecF with wild-type efficiency (Horii and Clark 1973; Lloyd et al. 1987). In vitro studies by Handa et al. (2009) showed that the RecJ nuclease and RecQ helicase can substitute for RecBCD

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at DSBs, leading to resection and RecF-mediated loading of RecA on ssDNA. The RecF pathway is therefore not restricted to SSG repair, but can also participate to DSB repair. Similarly, in bacteria devoid of RecB, such as *Deinococcus radiodurans*, the RecF pathway is employed to repair DSB (Bentchikou et al. 2010). In contrast, until recently, no evidence pointed towards a role for the RecBCD pathway in repairing SSGs, unless the gaps were converted to DSBs (Wang and Smith 1983).

The processing of DSBs and its genetic requirements have been widely explored through the study of biological processes such as conjugation or transduction since these processes initiate at DSBs. Moreover, the recent possibility to generate a unique DSB at a specific locus on the bacterial chromosome helped to clarify the mechanisms involved (Lesterlin et al. 2013; Cockram et al. 2015). But, our understanding of how SSGs are processed has remained relatively incomplete.

Our group recently developed a system to insert a single replication-blocking lesion into the chromosome of a living bacteria (Pagès et al. 2012; Naiman et al. 2014), thus allowing to study the repair of the SSG generated at the damaged site. By means of genetic markers placed at both the damaged and non-damaged strand, we were able to directly monitor sister chromatid strand exchange in the vicinity of the lesion site (Laureti et al. 2015). Using this system, we explored the repair of the SSG generated at a single UV lesion by monitoring the exchange of genetic markers between the damaged strand and its non-damaged homologous sister chromatid. We named this process homology-directed gap repair (HDGR).

We found that 90 % of cells repaired these SSGs by HDGR and these frequencies dropped to less than 10 % in the absence of RecA (Fig. 1). As expected, the inactivation of RecF led to a strong decrease of HDGR frequencies, with the majority of the colonies arising from “damaged chromatid loss” events in this strain (Laureti et al. 2015). Surprisingly, we observed a decrease in HDGR frequencies in a *recB* strain, suggesting an involvement of the RecBCD pathway in SSG repair.

It appears that the action of RecB does not occur through the conversion of the SSG into DSB. Indeed, if a DSB was formed and processed by RecBCD, it would lead to extended resection of the DNA end. The helicase activity of RecBCD unwinds an average of 30 kbp in vitro (Roman et al. 1992), and recent in vivo studies involving the generation of a single DSB confirmed a 30-kbp region of ssDNA coated by RecA (Cockram et al. 2015). Such resection would remove the markers we have placed at a short distance (between 100 and 800 bp) upstream of the lesion. Instead, the markers persist in the presence of RecBCD, and we were able to visualize the exchange of genetic markers between the damaged and non-damaged

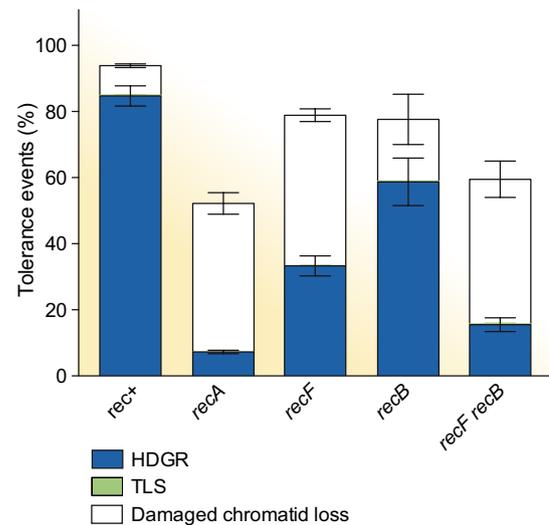


Fig. 1 The graph represents the partition of DNA damage-tolerance mechanisms for the TT(6-4) photoproduct in our parental strain (*uvrA*, *mutS*), and the different mutants impaired in homologous recombination: *recA*, *recF*, *recB*, and *recF recB*. The bars in blue represent the extent of SSG repair, also named homology-directed gap repair (HDGR). Adapted from Laureti et al.: the graph represents the average of the bypass of the lesion located on the leading and lagging strands

chromatid, evidencing genuine HDGR. Therefore, the RecBCD pathway could directly be involved in SSG repair. Interestingly, the inactivation of RecB leads to a decrease in HDGR even in the presence of a fully functional RecF pathway, supporting the view that the RecBCD pathways do not act as a backup in the absence of RecF (as RecF does in the absence of RecB), but is genuinely involved in SSG repair (Fig. 2). These findings raise the question of what dictates RecBCD versus RecF pathway choice for SSG. Could the extent of ssDNA at the SSG determine the pathway choice? It will be interesting to further explore this role of RecB. Are additional co-factors required to help RecB in SSG repair, or does RecB act alone in promoting the loading of RecA directly on SSB-coated ssDNA generated at the gap? Previous work revealed that in addition to the helicase and nuclease activity of RecBCD, the complex also facilitates the loading of RecA (Churchill et al. 1999) and that this activity is essential for HR (Arnold and Kowalczykowski 2000). While this activity was shown to be coupled to DNA unwinding in a substrate mimicking a DSB, it will be interesting to see if such RecA loading activity could also be observed on a gapped DNA.

Finally, our data show that in the absence of both RecF and RecB the frequency of SSG repair is as low as in a *recA* mutant. It appears therefore that the action of RecA is very limited in the absence of its key co-factors RecF and RecB. Interestingly, even in the absence of RecA, cells are still able to achieve some exchange of genetic information

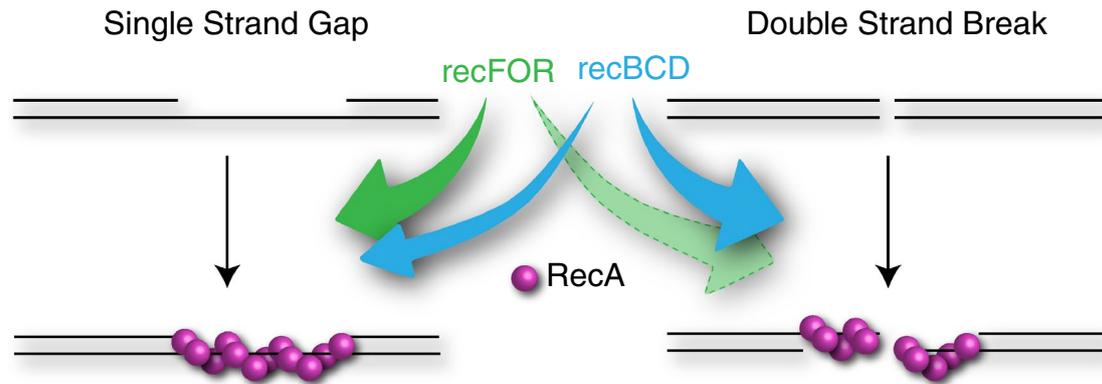


Fig. 2 In a normal situation, RecBCD pathway (*blue arrow*) is in charge of 99 % of DSB repair. In the absence of RecBCD, RecF pathway (*green arrow*) can substitute to RecBCD and process DSB

with the same efficiency. While the RecF pathway is responsible for a majority of SSG repair, recent data show that RecB also contributes to SSG repair

between the damaged and non-damaged strands. In the future, we will exploit our genetic system to further characterize the actors involved in RecA-independent SSG repair.

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