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Mini review

Early steps of double-strand break repair in *Bacillus subtilis*

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ABSTRACT

All organisms rely on integrated networks to repair DNA double-strand breaks (DSBs) in order to preserve the integrity of the genetic information, to re-establish replication, and to ensure proper chromosomal segregation. Genetic, cytological, biochemical and structural approaches have been used to analyze how *Bacillus subtilis* senses DNA damage and responds to DSBs. RecN, which is among the first responders to DNA DSBs, promotes the ordered recruitment of repair proteins to the site of a lesion. Cells have evolved different mechanisms for efficient end processing to create a 3′-tailed duplex DNA, the substrate for RecA binding, in the repair of one- and two-ended DSBs. Strand continuity is re-established *via* homologous recombination (HR), utilizing an intact homologous DNA molecule as a template. In the absence of transient diploidy or of HR, however, two-ended DSBs can be directly re-ligated *via* error-prone non-homologous end-joining. Here we review recent findings that shed light on the early stages of DSB repair in Firmicutes.

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Contents

1. Introduction 163
2. Tools to analyze the cellular response to DNA DSBs: from classical genetics to single molecule visualization 163
3. Bacterial chromosome dynamics under physiological conditions 165
4. DNA-damage responses 166
   4.1. Responses to damage without DNA breaks 166
   4.2. Do bacteria link DSB sensing to end processing? 166
   4.3. RecA phosphorylation or SsbA dephosphorylation might not control RecN levels 167
5. RecN is among the first responders to DNA DSBs 167
6. There are redundancies to resect the broken ends 168
   6.1. Initial end processing 168
   6.2. Long-range resection 169
7. HR *versus* NHEJ 171
8. RecN promotes tethering of ssDNA ends 171
9. Recombinase loading proceeds by different pathways 171
10. Conclusions and implications 172
    Conflict of interest 173
    Acknowledgements 173
    References 173

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1. Introduction

The faithful replication and maintenance of the genome is of primary importance for all living organisms. DNA damage is a serious threat to cellular homeostasis, and in a first step cells utilize specialized repair pathways to recognize the DNA damage and remove the lesion(s). There are various avenues to recognize a DNA lesion: a sensor protein can directly recognize the lesion, as with MutS, which binds to mismatched bases [1,2] or the recognition can be indirect by a protein·protein interaction, as with Mfd, which binds to RNA polymerase halted at a DNA damage site [2–4]. Simple chemical alterations of DNA bases are removed by base excision or nucleotide excision repair. Complex lesions, such as DNA interstrand/intrastrand crosslinks (ICLs) are corrected by nucleotide excision repair, and other coordinated pathways in eukaryotes [1,2,5–8]. If any of these steps fails, single-stranded (ss) DNA regions are exposed, and they are indirectly recognized and processed by the error free homologous recombination (HR) system. HR is a universal mechanism for restoring integrity, maintaining the stability and proper segregation of the genome. HR effectively re-establishes error-free strand continuity using the undamaged complementary strand as a template [9,10]. Among the numerous DNA lesions that occur naturally, or are induced by genotoxic agents, double-strand breaks (DSBs), which cause genetic instability, are arguably the most toxic lesions, leading to cell death if unrepaired. A DSB resulting from exogenous sources of DNA damage, such as ionizing radiation (IR), has two ends, whereas processing of a stalled replication fork or a DSB that arises at a collapsed replication fork has only one free end. The options available to repair a DSB differ depending on where and when it occurs – in front of the replication fork (in non-replicated DNA, in stationary cells or upon cell differentiation, *e.g.*, in spores), at the replication fork, or behind the replication fork in transient diploid DNA. DSB repair by HR in *Escherichia* has been studied extensively over five decades, but parallel studies in *Bacillus subtilis* are currently revealing some interesting departures from the *Escherichia coli* model system, as well as some analogies to eukaryotic cells [11].

The evolutionary distance between *B. subtilis* and *E. coli* is more than 1.5 billion years, which is a time divergence larger than the one between plants and animals, and is consistent with the differences in DNA replication, recombination, and repair between both bacteria [reviewed by 11–13]. In the following set of repair-related steps, some functions are highly conserved, but many differences will become apparent, some of which are elaborated in this review. First, in *B. subtilis* cells, one- and two-ended DSBs are mainly repaired by error-free HR if an intact copy of the broken chromosome segment is available as a template for DNA synthesis across the break, but two-ended DSBs are repaired by HR, or by error-prone non-homologous end joining (NHEJ) in the absence of transient diploidy [11,14–16]. Second, in *B. subtilis* PcrA (counterpart of *E. coli* UvrD [$UvrD_{Eco}$]) is essential, but $UvrD_{Eco}$ mutants are viable [17]. Third, the pairwise absences of RecG and RuvAB are synthetically lethal in *B. subtilis* [18,19], whereas in *E. coli* they are viable, albeit sensitive to DNA damage [20–25]. Finally, the decision at DNA damage sites differs between *B. subtilis* and *E. coli* cells. The absence of SOS induction does not affect the survival of *B. subtilis* cells with two-ended DSBs, whereas it drastically reduces the survival of *E. coli* cells [26]. The above differences between *B. subtilis* and *E. coli* make the former an attractive candidate to provide insight into the function of previously uncharacterized proteins, and to define the core components of the early steps of DSB repair. This is relevant because the detailed knowledge of crucial steps in any of the repair pathways is often still incomplete. This review focuses on aspects of the molecular mechanisms of the initial steps of DNA-damage response (DDR) and DNA DSB recognition and repair in *B. subtilis*. The reader can find detailed overviews on the initial steps of DSB in *E. coli* cells in recent authoritative reviews [20–25,27–29], as well as a comparison with other bacterial species [11]. Note that unless stated otherwise, the indicated genes and products are of *B. subtilis* origin. The nomenclature used to denote the origin of proteins from other bacteria is based on the bacterial genus and species (*e.g.*, *E. coli* RecA is referred to as $RecA_{Eco}$).

HR-mediated DSB repair can be subdivided into five discrete steps: (a) recognition of the break site and initial response to DNA damage; (b) end-processing at the break (generation of single-stranded (ss) DNA) and DSB "*coordination*"; (c) loading of the strand exchange protein RecA onto ssDNA; (d) strand exchange between broken and non-broken sister chromosomes, and formation or not of a Holliday junction (HJ); and (e) replication fork re-start, branch migration, and resolution/dissolution of the HJ, and chromosomal segregation. The early stage of DSB repair or pre-synaptic stage comprises the first three steps; of these, step (a) might be common for HR and NHEJ, but step (b) and (c) would direct the DSBs to be repaired by HR rather than by NHEJ because end resection, which is inhibitory to NHEJ, constitutes a critical control point in the pathway choice [11,30]. For simplicity in this review we focus on the early stage of two-ended DSB repair (steps a–c, Table 1).

2. Tools to analyze the cellular response to DNA DSBs: from classical genetics to single molecule visualization

Classical genetic studies were performed to classify the recombination genes, other than *recA*, within nine different epistatic groups (α to κ groups) based on sensitivity to different mutagenic agents: α (*recF*, *recO* and *recR*), β (*addA* and *addB*), γ (*recX* [*yfhG*]), δ (*recN*), ε (*recU*, *ruvA* and *ruvB*), ζ (*recJ*, *recQ* [*yocI*] and *recS* [*recQ or ypbC*]), η (*recG* [*ylpB*]), ι (sbcC and sbcD) and κ (*sbcE* [*yhaN*] and *sbcF* [*yhaO*]) epistatic groups [31–33]. The *recA*, *recF*, *recG*, *recJ*, *recN*, *recO*, *recQ*, *recR*, *recX*, *ruvA*, *ruvB*, *sbcC*, and *sbcD* genes have their counterparts in *E. coli* in genes with identical names, whereas the *addAB* and *recU* genes have their counterparts in the $recBCD_{Eco}$ and $ruvC_{Eco}$ genes, respectively [11]. The *recS*, *sbcE*, and *sbcF* genes have no obvious counterpart in *E. coli* [11]. There are additional functions that impact the cellular response to DSBs, namely the essential genes *dnaB*, *dnaD*, *dnaX*, *ssbA*, *polA*, *smc*, *scpA*, *and scpB*, and the non-essential *ku* (*ykoV*) and *ligD* (*ykoU*) gene [31]. The *dnaX* and *polA* genes have their counterparts in *E. coli* genes with identical names, whereas ssbA and *smc*, *scpA*, *and scpB* genes have their counterparts in the ssb_{Eco} and $mukBEF_{Eco}$ genes. The *dnaB*, *dnaD*, *ku*, and *ligD* genes have no obvious counterpart in *E. coli* (note that the hexameric replicative helicase $DnaB_{Eco}$ is not the counterpart of the *B. subtilis* protein with the same name) [31].

To understand the order of events during one- or two-ended DSB repair, and to study the spatio-temporal assembly of the recombinosome, different recombination and repair proteins have been tagged with a fluorescent protein [34] and characterized by multiple imaging techniques. The accumulation of these fused proteins might not be restricted to the DNA damage site itself, but rather spread out, flanking the DSB. To form microscopically visible foci, the local concentration of the repair proteins must be higher than 15–20 molecules, which is a possible caveat to this approach. In many conditions the DNA repair gene was fused with a gene coding for fluorescent protein (*e.g.*, GFP, CFP, YFP), and the respective wt gene was replaced by the fused gene at the wt locus, so that the fusion protein was regulated by the native promoter [19,32,35–40]. Whereas, when the fusion protein was not functional, its gene, under the control of an inducible promoter, was ectopically integrated, and both the wt and the fused protein were expressed [41].

Structural features of the repair process have also been characterized by single molecule imaging techniques such as electron microscopy (EM) and atomic force microscopy (AFM). The

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