DNA Repair xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Specificity in suppression of SOS expression by *recA4162* and *uvrD303*

Shawn C. Massoni, Steven J. Sandler*

Department of Microbiology, Morrill Science Center IV N203, University of Massachusetts at Amherst, Amherst, MA 01003, USA

ARTICLE INFO

Article history:
Received 29 January 2013
Received in revised form 29 August 2013
Accepted 4 September 2013
Available online xxx

Keywords:
SOS response
Homologous recombination
DNA repair
DNA replication

ABSTRACT

Detection and repair of DNA damage is essential in all organisms and depends on the ability of proteins recognizing and processing specific DNA substrates. In *E. coli*, the RecA protein forms a filament on single-stranded DNA (ssDNA) produced by DNA damage and induces the SOS response. Previous work has shown that one type of *recA* mutation (*e.g.*, *recA4162* (*I298V*)) and one type of *uvrD* mutation (*e.g.*, *uvrD303* (D403A, D404A)) can differentially decrease SOS expression depending on the type of inducing treatments (UV damage *versus* RecA mutants that constitutively express SOS). Here it is tested using other SOS inducing conditions if there is a general feature of ssDNA generated during these treatments that allows *recA4162* and *uvrD303* to decrease SOS expression. The SOS inducing conditions tested include growing cells containing temperature-sensitive DNA replication mutations (*dnaE486*, *dnaG2903*, *dnaN159, dnaZ2016* (at 37 °C)), a *del(polA)501* mutation and induction of Double-Strand Breaks (DSBs). *uvrD303* could decrease SOS expression under all conditions, while *recA4162* could decrease SOS expression under all conditions except in the *polA* strain or when DSBs occur. It is hypothesized that *recA4162* suppresses SOS expression best when the ssDNA occurs at a gap and that *uvrD303* is able to decrease SOS expression when the ssDNA is either at a gap or when it is generated at a DSB (but does so better at a gap).

1. Introduction

DNA damage-inducible responses are found in almost every organism. In eukaryotes, these are often regulated by the ATR and ATM kinases, which activate the signal transduction pathways that coordinate cell division and genome duplication [1]. In *Escherichia coli* (and many other bacteria [2]), the SOS response is regulated at the level of transcription by the RecA and LexA proteins [3–6]. While many studies on the SOS response have focused on its induction after treatment with DNA damaging agents such as mitomycin C or UV irradiation [7,8], induction of the SOS response also occurs during conjugation [9], cell envelope stress [10] and after treatment with β-lactam antibiotics [11,12]. The latter is of particular importance because induction of SOS produces mutagenic polymerases that then can increase the likelihood of cells becoming resistant to that antibiotic [13–15]. The SOS response also plays a role in persistence [16,17], regulation of integrons [18], the induction of bacterial programmed cell death through the activation of toxin–antitoxin systems [19], expression of some drug resistance determinants [20] and is crucial for the pathogenicity of some bacteria [21].

At homeostasis in log phase cells, LexA binds to sites in promoters of at least 40 genes repressing transcription [22,23]. It also binds at other sites on the chromosome not in promoter regions. The function of these sites, if any, is yet to be determined [24]. It is thought that the processing of DNA damage activates the SOS response by liberating regions of ssDNA to which RecA can bind and polymerize to form a nucleoprotein filament. This filament is an allosteric effector of LexA auto-proteolysis [25,26]. When the level of LexA decreases sufficiently in the cell, these promoters become active and increase the expression of the SOS genes, which aid in the cell's ability to survive the DNA damage. Eventually, as the damage is repaired, the amount of ssDNA shrinks and the level of LexA rises to turn off SOS and complete the cycle.

The SOS response has been most studied under conditions of external DNA damaging agents such as UV irradiation where there are typically many lesions per chromosome [7]. It is also known that replication forks routinely encounter "housekeeping" types of DNA damage [27]. These could include damaged bases, nicks in the DNA or protein blocks [28–30]. Although RecA participates in repair of these types of lesions through its ability to form a RecA-DNA filament, it is clear that the SOS response is not usually induced. This is best demonstrated by the observations that about 15–25% of a population of log phase cells have recombination structures at any one time [31–33], yet less than 1% are induced for SOS [34,35]. Recently, it has been shown that *radA*, the amount of RecA in the cell, and in some cases *recX*, prevent these RecA filaments from inducing the SOS response when presumably fixing housekeeping types of damage [31]. Thus, the cell has the ability to discriminate between types and/or amounts of DNA damage to induce the SOS

* Corresponding author. Tel.: +1 413 577 4391; fax: +1 413 545 1578.
E-mail address: sandler@microbio.umass.edu (S.J. Sandler).

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http://dx.doi.org/10.1016/j.dnarep.2013.09.003

Table 1
Summary of the DNA replication mutants used in this study.

Mutant gene (amino acid change)	Name of protein	Function of protein in DNA replication	Defects in DNA replication and or phenotypes for this allele	References
dnaE486 (S885P)	α subunit of DNA polymerase III	Catalytic subunit of DNA polymerase III holoenzyme	Mutator phenotype (Pol V-dependent) and whose interactions with the β clamp may be compromised at high temperatures	[41,72–74]
dnaG2903[a] (E567K)	Primase	Primer DNA replication on the lagging strand	Inviable at 42 °C, however, no effect on ongoing DNA and RNA primer synthesis. This mutation is located in a poorly conserved region of *dnaG* that mediates interactions with DnaB	[75–78]
dnaN159 (G174A)	β clamp	Processivity subunit	Compromised in interactions with the α subunit at 42 °C and is 3-fold more UV^s than wild type	[66,79]
dnaZ2016[b] *(aka dnaX2016)* (G118D)	γ subunit clamp loader	Stabilizes interactions between Pol III and DnaB and loads β clamp	Temperature sensitive for DNA replication and cell division (reversible). Defective in ATPase activity and β clamp placement at high temperatures	[80–84]
del(polA)501	DNA polymerase I	Okazaki fragment maturation	Inviable in rich medium, UV^s, grows poorly	[34,85,86]

[a] Originally known as *dnaP* for phenethyl alcohol resistance [78].
[b] Originally known as *dnaH* [82].

response. Presumably this depends on when and where RecA can polymerize on ssDNA to produce filaments as well as their duration in the cell and their accessibility to LexA.

Historically, research on SOS regulation has focused on mutants that are defective in this regulation. Two types of regulatory mutants have been described for *recA*. The first type constitutively expresses SOS in the absence of external DNA damage. Several of these types of mutants have been described (reviewed in [36]). It has been shown for two of these mutants, *recA4142* (F217Y) and *recA730* (E38K), that while they both cause SOS constitutive (SOS^C) expression, they do so through different mechanisms [37–39]. SOS^C expression in *recA4142* mutants depends on several genes: *recBCD*, *ruvAB*, *recJ* and *sbcB* [37]. It was proposed that RecBCD loads RecA4142 onto the ends of a replication fork that has been reversed by RuvAB and tailored by RecJ and SbcB. SOS^C expression in a *recA730* mutant is not dependent on any of these genes. RecA730 is thought to bind to ssDNA on the lagging strand at a replication fork, although there is no direct data supporting this model.

Another type of SOS regulatory mutant that has been isolated in *recA* is one that genetically suppresses the SOS^C expression of *recA4142* and *recA730*. Two alleles of this type, *recA4162* (I298V) and *recA4164* (L126V), have been isolated ([39] and references therein). They are able to inhibit the SOS^C expression of *recA730* and *recA4142* both intragenically (*in cis*) and extragenically (*in trans*) [39]. This inhibition depends on both *uvrD* and *recX*. These two proteins are known to destabilize RecA-DNA filaments under certain conditions both *in vivo* and *in vitro* [40–46]. It was hypothesized that RecA4162 and RecA4164 better respond to and/or recruit UvrD and RecX to destabilize the filaments and thus lower SOS expression. It was also shown that *recA4162* and *recA4164* mutants were Rec^+ UV^R and, importantly, were able to induce the SOS response after UV treatment in a manner similar to wild type [39]. The fact that *recA4162* is able to induce the SOS response under some conditions, but not others, is the impetus for this study. Since *recA4162* and *recA4164* behave in a similar fashion, only *recA4162* will be further considered here.

Besides *recA* mutations, other antagonists of SOS expression exist that affect the ability of the cell to induce SOS in a *recA730* mutant. It was shown that *uvrD303* could reduce SOS expression in a *recA730* mutant background and after UV irradiation [47]. *uvrD303* was constructed by Kushner and colleagues [48]. It has two point mutations (D403A, D404A) located in the 2B subdomain of the protein. UvrD is nearly structurally identical to the Rep helicase [49,50]. In Rep, the 2B domain is not essential for helicase activity [51]. The 2B domain can rotate (by about 130°), is coupled to nucleotide and DNA binding, and is hypothesized to be important for regulation of helicase activity [52,53]. Since UvrD303 has up to a 10-fold higher helicase activity than wild type depending upon the substrate tested, it was characterized as a "hyperhelicase" [48]. The *uvrD303* mutant is recombination-deficient, UV-sensitive, has lower mutability and can decrease the levels of RecA activity in the cell via a proposed direct interaction between the C-terminus of UvrD303 and RecA [47,48]. The only instance reported thus far where UvrD303 is unable to decrease constitutive SOS expression is in a *recA4142* mutant [47]. Hence, it would seem that *uvrD303* has some specificity.

Several DNA replication mutants cause SOS expression in the absence of external damage (Table 1). These mutants include *dnaE486*, *dnaG2903*, *dnaN159* and *dnaZ2106*. These genes encode several of the proteins in the sub-assemblies of a replication fork. All of these mutants are viable at 30 °C and inviable at 42 °C. All but *dnaG2903* inhibit DNA replication at the non-permissive temperature (Table 1). Of key importance to this study is that all mutants tested show high levels of SOS expression in the absence of external damage at the semi-permissive temperature of 37 °C (Table 1). While the reason for this is not known, it has been hypothesized that the DNA replication fork is destabilized and/or disabled and this creates ssDNA to which RecA can bind and induce the SOS response.*polA501* mutations also have high levels of SOS expression [34]. The reason for this could be at least two fold. First, *polA* mutants have defects in processing Okazaki fragments. These mutants are likely to have many more gaps in the newly synthesized lagging strand DNA than wild type. It is also known that *polA501* is synthetically lethal with *recA* and *recB* mutations [54–57]. Therefore it is likely that some of these gaps may be slow to be repaired (repair of gaps required *polA*) and could be converted into Double Strand Breaks (DSBs) by either the action of nucleases or another round of DNA replication [58,59]. Thus *polA501* mutants could have either gaps or DSB that could be bound by RecA to trigger SOS induction.

In this study we asked whether *recA4162* or *uvrD303* can lower SOS expression in strains that have high levels of SOS expression due to defects at the replication forks or a DSB produced by I-*Sce*I. It is shown that *uvrD303* decreases SOS expression to a large degree (equal to *recA4162*) in all the DNA replication mutants. It also decreases SOS in a *polA501* mutant and after I-*Sce*I treatment producing a DSB, but to a lesser extent. *recA4162*, however, only inhibits SOS expression in the four temperature sensitive DNA replication mutants. It does not suppress expression in the *polA501* strain or

Please cite this article in press as: S.C. Massoni, S.J. Sandler, Specificity in suppression of SOS expression by *recA4162* and *uvrD303*, DNA Repair (2013), http://dx.doi.org/10.1016/j.dnarep.2013.09.003