DNA Damage Responses in Prokaryotes: Regulating Gene Expression, Modulating Growth Patterns, and Manipulating Replication Forks

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Recent advances in the area of bacterial DNA damage responses are reviewed here. The SOS pathway is still the major paradigm of bacterial DNA damage response, and recent studies have clarified the mechanisms of SOS induction and key physiological roles of SOS including a very major role in genetic exchange and variation. When considering diverse bacteria, it is clear that SOS is not a uniform pathway with one purpose, but rather a platform that has evolved for differing functions in different bacteria. Relating in part to the SOS response, the field has uncovered multiple apparent cell-cycle checkpoints that assist cell survival after DNA damage and remarkable pathways that induce programmed cell death in bacteria. Bacterial DNA damage responses are also much broader than SOS, and several important examples of LexA-independent regulation will be reviewed. Finally, some recent advances that relate to the replication and repair of damaged DNA will be summarized.

Since the publication of *DNA Repair and Mutagenesis* in 2006 (Friedberg et al. 2006), our understanding of bacterial DNA damage responses has progressed significantly. Some studies have refined known pathways and filled in important details, whereas other studies have uncovered surprising new pathways such as bacterial programmed cell death and a form of replicative repair that reconstitutes severely shattered genomes. This review will focus on these recent advances, with only limited discussion and citation to work that precedes the 2006 tome.

THE SOS RESPONSE

As scientists have studied the SOS response in *Escherichia coli* and other bacteria over the last decade, our understanding of SOS has changed in unexpected ways and the pathway has been found integrated in diverse cellular processes. Our understanding of the mechanism of SOS induction has also advanced in recent years, including a view of induction at the single-cell level. A surprising finding is that the functions of the SOS pathway are not uniform between bacterial species, and so the view of SOS as

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purely a DNA damage response pathway is insufficient. Furthermore, SOS is not the major DNA damage response pathway in some bacterial species (see “SOS-Independent DNA Damage Responses” below). From a practical viewpoint, recent findings also uncover clinical significance of the SOS pathway in the response to diverse antibiotics and also in the spread of virulence and drug-resistance genes. The reader is referred to several excellent recent reviews on various aspects of the SOS response (Michel 2005; Aertsen and Michiels 2006; Kelley 2006; Erill et al. 2007; Butala et al. 2009; Ayora et al. 2011) and to Fuchs and Fujii (2013) on bacterial translesion synthesis (TLS) polymerases for a discussion of this aspect of the SOS response.

SOS Response in *E. coli*

The Basic Circuitry of the SOS Response

The SOS response in *E. coli* coordinates the cells response to DNA damage by the action of the LexA repressor (Fig. 1) (for review, see Friedberg et al. 2006, and above-cited reviews). LexA pro-

tein in its native state binds to the promoter region of multiple SOS genes during normal growth limiting their expression levels. Upon DNA damage, the RecA protein is activated to become a coprotease by formation of a filament of RecA on single-stranded DNA (ssDNA), which facilitates the self-cleavage reaction of LexA. Thus, as LexA repressor levels decrease, expression levels of SOS regulon genes increase. The products of SOS regulon genes are involved in specific DNA repair mechanisms, tolerance of DNA damage, and induced delay of the cell cycle.

LexA also regulates transcription of both its own gene and the *recA* gene, which is important for the overall function of the circuit. Increased RecA protein levels after DNA damage promotes recombinational repair and effective cleavage of LexA, whereas increased LexA expression allows the SOS response to be rapidly shut off when the inducing signal of ssDNA wanes. Activated RecA protein also serves as coprotease for the bacteriophage λ repressor and for UmuD, an *E. coli* protein involved in DNA damage tolerance and TLS (as well as homologs of both proteins).

Figure 1. The *E. coli* SOS response. The basic circuitry of the *E. coli* SOS response is summarized. RecA protein-DNA filaments serve as a coprotease to activate self-cleavage of the LexA protein, the global repressor of SOS. As described in the text, RecA can also activate self-cleavage of UmuD and the phage λ repressor.

Genome-scale approaches have refined our knowledge of the *E. coli* genes induced by DNA damage in both SOS-dependent and -independent modes (reviewed by Michel 2005; Kelley 2006; Friedberg et al. 2006). Microarray studies revealed SOS-inducible genes that were largely expected from prior genetic and molecular studies in *E. coli* (Courcelle et al. 2001; Zheng et al. 2001; Khil and Camerini-Otero 2002). Furthermore, a chromatin immunoprecipitation (ChIP) on chip study confirmed 25 expected LexA binding sites, and also uncovered five new sites with a LexA consensus sequence and 19 apparent LexA binding sites without any obvious LexA motif (Wade et al. 2005).

As described in detail by Friedberg et al. (2006), some genes of the *E. coli* SOS pathway, such as *sulA* and *umuDC*, are induced only late and/or with high levels of DNA damage. The molecular explanation is that increasing levels of DNA damage leads to progressively increasing RecA-promoted LexA proteolysis and thus decreasing LexA concentrations. Thus, a relatively high level of DNA damage is necessary to achieve the very low LexA concentrations that allow induction of genes such as *sulA* and *umuDC*, with their very strong LexA binding sites. This arrangement allows the SOS response to be graded, for example, facilitating DNA repair without blocking cell division or inducing TLS polymerase activity at low levels of DNA damage.

How Exactly Is SOS Induced?

Recent studies have brought significant molecular detail to our understanding of how the SOS response is induced after DNA damage. Biochemical analyses showed that only the DNA-free form of LexA is cleaved by activated RecA protein (Butala et al. 2011). This study has implications that relate the relative strength of LexA binding sites to the sequential order of induction of genes during the damage response. Using substitution mutants of RecA, Gruenig et al. (2008) provided evidence that RecA filament formation, but not ATPase activity, are needed for RecA-induced LexA cleavage. Also relevant to the induction mechanism, muta-

tional analyses of RecA filament surface residues defined a discrete LexA binding region (Adikesavan et al. 2011).

Bulk approaches for studying SOS induction have limitations, including the inability to assess whether all cells in a population induce the pathway similarly. Additional insight can be obtained at the single-cell level, using fusion constructs in which the green fluorescent protein (GFP) fluorescent reporter is coupled to either an SOS-inducible promoter or to the RecA protein itself. One new insight obtained from this approach relates to the subcellular localization of recombination proteins. Prior studies in eukaryotic cells revealed DNA damage-induced recombination protein foci in cell nuclei, which were implicated as recombination repair factories (Lisby et al. 2001; for review, see Lisby and Rothstein 2009). Similar structures were discovered in *E. coli* using a RecA-GFP fusion allele (Renzette et al. 2005). These investigators found that normal undamaged *E. coli* cells often displayed one or even multiple foci, about half of which were inferred to contain DNA (based on sensitivity to DAPI [4',6-diamidino-2-phenylindole] treatment) and half not (presumed to be RecA storage structures). A RecA substitution mutant that does not form storage structures in vitro but is otherwise normal (Eldin et al. 2000) provided a simpler tool because a GFP fusion to this mutant RecA showed only DNA-containing (DAPI-sensitive) foci (Renzette et al. 2005). Importantly, DNA damage induced by UV treatment led to an increased number of foci, and the foci relocated to a position near the cell center. The new position at cell center was proposed to coincide with the sites of replication machinery, arguing that the recombination foci were actively involved in dealing with DNA damage at the replication fork (see "Prokaryotic Strategies for Replicating Damaged DNA" below).

A second important insight emerged by analyzing the time course of induction of GFP fused to SOS promoters. Friedman et al. (2005) showed cascading waves of SOS induction in single cells during a post-UV incubation period. These waves were not previously detected in bulk cultures because of asynchrony between

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individual cells in a population. The investigators speculate that resumption of DNA replication may cause one of the later waves, and presented evidence that UmuD cleavage plays an important regulatory role (see discussion of checkpoint function of UmuDC in “Bacterial Damage Response Checkpoints” below). A quantitative analysis of the dynamics and mathematical modeling of the cascading waves of induction was provided in the later work of Shimoni et al. (2009).

DNA Damage Is Not the Only Inducer

A much broader array of conditions, well beyond DNA damage and replication fork failure, have been shown capable of inducing the SOS response. One natural situation that can induce SOS is the introduction of ssDNA into a recipient cell during a conjugation event (Bailone et al. 1988; Golub and Panzer 1988; Bagdasarian et al. 1992; Petrova et al. 2009; Baharoglu et al. 2010). Indeed, many plasmids encode proteins that bind and inhibit RecA to prevent SOS induction during conjugation, such as the PsiB protein of F plasmid. As already discussed by Friedberg et al. (2006), certain β -lactam antibiotics that inhibit cell-wall synthesis can also induce SOS in *E. coli*, but via a distinct pathway involving the DpiBA two-component system (Miller et al. 2003, 2004; also see Aertsen and Michiels 2006; Maiques et al. 2006). Induction by a broad array of antibiotics and the very act of conjugation has important implications for spread of antibiotic resistance and virulence genes (see “A Broader View of the Physiology and Consequences of the SOS Response” below).

Nature of Spontaneous SOS in Untreated Cells

The single-cell approach has also been useful for analyzing the low level of SOS expression that can be detected in untreated cells. McCool et al. (2004) fused a GFP fluorescent reporter to the *sulA* gene promoter and analyzed SOS induction in single cells by fluorescence microscopy. Untreated wild-type cells showed a small fraction of cells with significant levels of SOS induc-

tion, showing that the overall low level of SOS was caused by a small subset of induced cells that had apparently suffered some kind of spontaneous DNA damage (McCool et al. 2004). Interestingly, the same pattern held for various mutants that had been classified as partially constitutive for SOS (e.g., *priA*, *uvrD*, *dam*, and *rnhA*)—each of these showed a larger subpopulation of cells that were induced rather than a uniform level of induction in all cells. Pennington and Rosenberg (2007) continued the analysis of untreated wild-type cells and found that the fraction of induced cells was significantly lower than expected from previous studies; they attributed SOS induction to cells that suffer double-strand break (DSB) formation during the process of DNA replication (Pennington and Rosenberg 2007).

The graded response of SOS genes to LexA concentrations was noted above. The same kind of gradation has now been observed in single-cell studies of spontaneous SOS induction in *E. coli*. GFP fusions to the *recA* or *lexA* promoters resulted in more than 1% of cells showing spontaneous induction, whereas <0.1% of cells with an *umuDC* fusion were induced (Kamenšek et al. 2010). Thus, the severity of the DNA damage in those few unfortunate cells that require SOS induction apparently varies greatly, with a small subset apparently suffering fairly catastrophic levels of damage. It will be very interesting to figure out what kind of damages lead to the two (or perhaps more) different induction states.

Diversity in the SOS Response between Bacterial Species

Diversity in the SOS Circuit

Interesting surprises have arisen from studies of SOS in diverse bacteria. The genes induced by the SOS response in bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, analyzed with global approaches, show significant overlap but some divergence from those induced in *E. coli* (Courcelle et al. 2001; Au et al. 2005; Cirz et al. 2006, 2007; Goranov et al. 2006). There is diversity even considering genes whose products are involved

in DNA damage repair or tolerance, and only a small subset of the *E. coli* SOS-inducible genes are also in the *B. subtilis* SOS regulon. Thus, the precise functions of SOS-induced genes appear to be fine-tuned to the biology of the organism. Erill et al. (2007) discusses the evolution of the SOS system in detail.

Additional distinctions from the classical *E. coli* system support the view that the SOS system is fine-tuned to the biological system. First, in *E. coli*, LexA acts solely as a repressor protein, inhibiting gene expression in its uncleaved state. Several examples of positive regulation by LexA have now been identified in diverse bacterial species (Tapias et al. 2002; Gutekunst et al. 2005; Jochmann et al. 2009; Smollett et al. 2012). Second, the *Mycobacterium tuberculosis* LexA protein binds to the regulatory region of two genes encoding small RNAs (Smollett et al. 2012). Third, unlike in *E. coli*, aminoglycosides, tetracycline, and chloramphenicol all induce SOS in *Vibrio cholerae* (Baharoglu and Mazel 2011).

Although older literature suggested that *Neisseria gonorrhea* lacks an SOS system (Black et al. 1998; Davidsen and Tønnum 2006), Schook et al. (2011) recently identified a LexA homolog encoded by gene ng1427. This LexA homolog regulates DNA repair (RecN, in particular) and can apparently be cleaved by RecA. Interestingly, the response is derepressed by reactive oxygen, possibly via a direct oxidation/reduction cycle, and so the induction pathway appears to be novel.

RecA Focus Formation Depends on DNA Replication in *B. subtilis*

The relationship of DNA damage, RecA foci, and SOS induction was explored in *B. subtilis* (Simmons et al. 2007; Simmons et al. 2009; for review, see Ayora et al. 2011). RecA focus formation was shown to be dependent on DNA replication, even when the inducing lesion was a DSB. Focus formation did not require the induction of RecA protein levels, so redistribution of existing protein was apparently sufficient. Focus formation was also clearly distinguished from SOS in this system because either ionizing

radiation or direct DSB induction led to foci in virtually every cell, but only a small subset of the cells were found to be induced for SOS (Simmons et al. 2009). In contrast, ionizing radiation induces foci formation in virtually every *E. coli* cell. These results parallel sensitivity results because SOS-noninducible *E. coli* are hypersensitive to ionizing radiation, but noninducible *B. subtilis* are unaffected. From all of these results, it is clear that *B. subtilis* has substantial and efficient repair pathways without SOS induction, and that RecA focus formation is mechanistically distinct from SOS induction (also see Centore et al. 2008; Massoni et al. 2012).

A Broader View of the Physiology and Consequences of the SOS Response

The SOS response is now recognized to play a major role in the generation and spread of antibiotic resistance and other clinically relevant events in bacteria, and is clearly an important factor in evolution of bacterial species and species variants. As reviewed by Kelley (2006) and Erill et al. (2007), subinhibitory levels of antibiotics can induce SOS leading to horizontal gene transfer, spread of antibiotic resistance, and induction of virulence factors.

SOS and Horizontal Gene Transfer

The role of SOS in horizontal gene transfer is widespread and quite remarkable. Starting with the classic example of phage λ , it has long been known that SOS induction results in reactivation of many lysogenic prophages, contributing to the spread of prophage-borne genes including virulence genes (see Friedberg et al. 2006; Goerke et al. 2006; Kelley 2006). One of the most dramatic examples of the impact of SOS-inducible phages involves a *V. cholerae* prophage that encodes cholera toxin; SOS induction of phage CTX ϕ greatly augments toxin production/release and leads to dissemination of the prophage (Quinones et al. 2005; Waldor and Friedman 2005).

In a parallel fashion, SOS has now been shown to activate conjugative transposons, also called integrative conjugative elements (ICEs).

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On induction, ICEs efficiently transfer from donor to recipient cells, sometimes of different species, carrying genes for antibiotic resistance, pathogenesis, alternative carbon metabolism, and symbiotic relationships (depending on the element; Wozniak and Waldor 2010). Although multiple induction mechanisms exist, the SOS response has been shown to induce ICEs including the SXT element of *V. cholera* (Beaber et al. 2004), the ECEBs1 element of *B. subtilis* (Auchtung et al. 2005), enteric IncJ elements (McGrath et al. 2005), ICEst1 and ICEst3 from *Streptococcus thermophilus* (Bellanger et al. 2007, 2008), and probably the RD2 element found in group A *Streptococcus* (Sitkiewicz et al. 2011).

The widespread role of SOS in horizontal gene transfer is also confirmed by its effects on pathogenicity islands. For example, *S. aureus* island SaPIbov1 excises and replicates on SOS activation of coresident prophages, which can then package the island into phage-like particles at high efficiency (Ubeda et al. 2005; Ubeda et al. 2007; Tormo et al. 2008). The pathway was shown to work with multiple pathogenicity islands, and clinical strains showed the same phenomenon with resident prophages and islands.

SOS and Antibiotic Resistance

SOS contributes to antibiotic resistance not just by stimulating gene transfer events, but also by inducing mutation and genomic rearrangements (also see a review on TLS polymerases by Fuchs and Fujii 2013). For example, Cirz et al. (2007) showed that the generation of antibiotic resistance in *S. aureus* in vitro required the SOS response and proposed that up-regulation of the tricarboxylic acid cycle and mutagenic TLS polymerases was involved (also see Kohanski et al. 2010).

Generation of antibiotic resistance from SOS can also result from induction of recombination events. In one prominent example, SOS induction was shown to induce expression of integrase recombination protein that rearranges integrons, thereby activating previously silent cassette genes including those encoding antibiotic resistance proteins (Guerin et al. 2009).

This is a potent mechanism for generation of antibiotic resistance in response to drug treatments, and also as a result of transient SOS induction in response to incoming ssDNA during conjugation or transformation (Baharoglu et al. 2010, 2012). Indeed, this molecular mechanism has been documented to contribute to resistance in a clinical setting. A strain of *P. aeruginosa* highly resistant to a third-generation cephalosporin emerged in a hospital patient at the University of Washington, and the strain went on to become epidemic within the hospital (Hocquet et al. 2012). The original patient had been previously treated with cephalosporin and metronidazole, and these investigators showed that the latter drug induces SOS. In turn, SOS induction in this organism activates the integrase IntI1, which apparently led to the cassette rearrangement that activated high-level expression of the β -lactamase.

A related concern is that SOS induction has now been shown to directly up-regulate the expression of certain antibiotic resistance genes (Da Re et al. 2009; Wang et al. 2009). The widespread plasmid-borne *qnr* genes encode proteins that provide resistance to the quinolones by direct binding to the quinolone target DNA gyrase. The *qnrB2* gene from a *Salmonella enterica* integron, along with other *qnrB* genes, have been shown to be under SOS control, with a conserved LexA binding site in their promoters (Da Re et al. 2009; Wang et al. 2009). Quinolones are potent inducers of SOS, and therefore this regulation mechanism can be directly detrimental to antibiotic therapy.

SOS and Bacterial Pathogenesis

As reviewed by Kelley (2006) and Erill et al. (2007), SOS also plays a direct role in bacterial pathogenesis because certain virulence genes in diverse bacteria are part of the SOS regulon. This creates a perilous situation in which either treatment with antibiotics or even the natural defense mechanisms of the host can induce virulence factor production in the pathogen (also see a recent study on role of SOS during entry of uropathogenic *E. coli* in a mouse model in Li et al. 2010).

CHECKPOINTS AND PERSISTERS AND APOPTOSIS, OH MY!

DNA damage responses including SOS are also tightly connected to cell growth inhibition and cell death events, and these also have profound implications for antibiotic action. Studies in the last several years have uncovered surprising complexities including striking modulations in cell growth and death pathways that impact survival after DNA damage and antibiotic treatments (Fig. 2).

Bacterial Damage Response Checkpoints *SulA* and Cell Division Checkpoints

It has long been appreciated that SOS induction of the *E. coli* SulA protein causes a cell-cycle

checkpoint by inhibiting cell division, acting at the level of FtsZ polymerization, and thereby providing the cell more time to repair DNA damage (see Friedberg et al. 2006). Recent evidence suggests that the SulA checkpoint in *E. coli* may be augmented by other checkpoint mechanisms (see below).

A cell-cycle checkpoint that acts directly on the cell division process, but in a manner distinct from SulA has been uncovered in *Caulobacter crescentus*, which has a eukaryotic-like cell cycle. DNA damage in this bacteria leads to an SOS response that includes the 29-amino acid SidA protein, which inhibits cell division by interacting with the FtsW/FtsN proteins and preventing final cytokinetic ring constriction (Modell et al. 2011).

Figure 2. Factors implicated in DNA damage-inducible modulation of bacterial cell growth and death. Proteins and other factors that have been implicated in checkpoint induction, persister cell formation, and programmed cell death are depicted. Subsets of these proteins may act in distinct pathways; the diagram does not imply that all proteins next to an arrow act together. See text for details.

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Toxin–Antitoxin Pairs as Checkpoints

One possible checkpoint mechanism involves the action of a toxin–antitoxin (TA) system (for reviews, see Gerdes et al. 2005; Magnuson 2007; Yamaguchi et al. 2011). Several toxin genes are induced during an SOS response, although the physiological impacts of most are yet to be deciphered (see Kawano et al. 2007; Magnuson 2007; Unoson and Wagner 2008; Yamaguchi et al. 2011; Gerdes and Maisonneuve 2012). An interesting example involves the *E. coli* *yafN* and *yafO* genes, which are downstream of *dinB*, and like *dinB*, up-regulated during an SOS response (Singletary et al. 2009). Although not proven, the investigators suggest that the toxin YafO delays reentry into the cell cycle following an SOS response, acting as a checkpoint to allow maximal repair of the damage. YafO was recently shown to be a ribosome-associated RNase that inhibits protein synthesis via mRNA cleavage (Zhang et al. 2009).

TLS-Related Checkpoints

E. coli TLS polymerases Pol IV (DinB) and Pol V (UmuD₂C) have both been linked to a possible checkpoint-like function relating to the SOS response (also see review on TLS polymerases by Fuchs and Fujii 2013). As described in detail by Friedberg et al. (2006), the intact form of UmuD together with UmuC appears to function in a damage-inducible checkpoint. RecA-dependent cleavage of UmuD late in the SOS response can generate the active Pol V (UmuD₂C), but this is apparently not the only function of this protein. Striking evidence for a possible checkpoint function is that expression of noncleavable UmuD (together with UmuC) increases survival after UV damage by delaying the resumption of DNA replication, which apparently allows more time for excision repair to fix UV-induced lesions (Opperman et al. 1999). Furthermore, separation-of-function mutations have been isolated to distinguish the checkpoint function and TLS polymerase activity (Sutton and Walker 2001).

Interestingly, some parallel evidence suggests that DinB, which encodes the TLS poly-

merase Pol IV, might also be a checkpoint effector. Overexpression of DinB was shown to rapidly block replication fork movement, and mutations in DinB differentially affected this fork blockage activity and TLS activity (Uchida et al. 2008). The DinB protein was also shown to displace Pol III from active replisomes in vitro, providing a molecular explanation for the in vivo fork blockage (Furukohri et al. 2008; Uchida et al. 2008). Further research is clearly needed to elucidate the physiological significance of the apparent checkpoint activities of UmuDC and DinB, both of which are induced by SOS.

Checkpoints Relating to Stringent Response

Two other checkpoint-like responses have been linked to the stringent response and are related to DNA replication inhibition (for review of the stringent response, see Potrykus and Cashel 2008; Srivatsan and Wang 2008; Battesti et al. 2011; Dalebroux and Swanson 2012). First, mutations in the *E. coli* *obgE* gene were shown to cause a dramatic hypersensitivity to DNA replication inhibitors, leading to a model in which the ObgE GTPase acts as a replication checkpoint protein of some kind (Foti et al. 2005). Based on synergy with recombination proteins, the investigators proposed that ObgE action prevents the accumulation of regressed forks and DNA breaks during the inhibition of replication. Subsequently, ObgE was found to bind ppGpp at physiologically relevant concentrations in vitro, and inactivation of ObgE activity was shown to increase the pppGpp to ppGpp ratio in vivo during the stringent response (Persky et al. 2009).

In a second and perhaps related case involving *B. subtilis*, induction of the stringent response to starvation was shown to rapidly inhibit DNA replication (Wang et al. 2007). Strikingly, the in vivo replication block was traced to a direct inhibitory effect of (p)ppGpp on DNA primase activity. The arrested replication forks did not recruit RecA protein, indicating that the mechanism of fork stalling was benign and presumably protective against genotoxic or potentially lethal fork processing events (Wang

et al. 2007). *E. coli* primase is also inhibited by (p)ppGpp, and the investigators speculated that the above ObgE effects might be exerted through primase inhibition (Maciej et al. 2010; also see Kanjee et al. 2012).

Another interesting connection to the stringent response involves the protein IraD, an anti-adaptor that inhibits the proteolysis of the stress-response sigma factor RpoS. *E. coli iraD* or *rpoS* knockout mutants are hypersensitive to azidothymidine, hydrogen peroxide and phleomycin, agents that cause DNA damage or inhibit DNA replication (Merrikh et al. 2009). IraD is induced after DNA damage in a LexA-independent fashion, and its induction stabilizes RpoS against degradation (Zheng et al. 2001; Merrikh et al. 2009). These and other results argue that the RpoS-based stress response, via IraD induction, acts synergistically with the SOS response in survival after DNA damage or replication inhibition (Merrikh et al. 2009). The proteins induced during the stress response that promote survival remain to be determined.

SOS Induction of Persister Cells that Survive Drug Treatment

Although inhibition of cell division may in certain cases serve a checkpoint response, giving cells more time to repair damage, it can also (or instead) have a dramatic population effect of inducing so-called persister cells (for recent reviews, see Lewis 2010; Gerdes and Maisonneuve 2012). Persisters are dormant variants of normal cells that are highly tolerant to antibiotics, but usually formed in only tiny fractions of a cell population. Certain TA pairs are crucial in the formation of persister cells that resist antibiotic treatment, and one in particular plays a specific and dramatic role during the SOS response (Magnuson 2007; Tsilibaris et al. 2007; Dörr et al. 2010; Lewis 2010; Yamaguchi et al. 2011).

Dörr et al. (2009) found that SOS induction increases the fraction of persisters able to survive antibiotic treatment. Thus, preexposure to a low level of ciprofloxacin was found to increase survival to higher levels of the drug. Deletions of the SOS-inducible *tisAB* TA pair dra-

matically reduced the fraction of persister cells, whereas deletion of other LexA-box-containing TA gene pairs had no effect (Dörr et al. 2010; Lewis 2010). Importantly, the adaptive response of pretreatment with a low level of drug was also abolished in the *tisAB* knockout (Dörr et al. 2010). Thus, SOS induction significantly increases persister cell formation by a TisB-dependent mechanism that turns out to involve membrane binding and disruption of the proton motive force (Unoson and Wagner 2008; Lewis 2010).

Bacterial Programmed Cell Death (PCD)

A number of recent studies suggest that bacteria undergo PCD under particularly stressful conditions, including treatment with agents that damage DNA and/or inhibit replication. At least one of these pathways bears striking resemblance to eukaryotic apoptosis. As will be described, however, contrary evidence also exists concerning some of these proposed pathways and more experiments are needed to clarify the extent and impact of bacterial PCD pathways.

MazF and PCD

The earliest studies that hinted at bacterial PCD involved the addiction modules that consist of TA pairs encoded by plasmids (for reviews, see Gerdes et al. 1990; Jensen and Gerdes 1995; Engelberg-Kulka and Glaser 1999; Gerdes et al. 2005). In this case, whenever a daughter cell arises without a copy of the plasmid because of a defective segregation event, the antitoxin decays faster than the toxin and the dominant toxin kills the cell (or at least arrests its growth). This is a sensible mechanism for plasmids to enforce addiction on their host, but then it is surprising that so many TA systems were found in chromosomal locations (see Magnuson 2007, for excellent discussion of possible TA functions).

One such chromosomal system that is widely conserved and extensively studied is the MazEF pair. Reports from one group argued that MazF toxin induction triggers a PCD path-

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way (Sat et al. 2001; Hazan et al. 2004), however, others instead detected reversible cell growth inhibition (Pedersen et al. 2002; Christensen et al. 2003; Tsilibaris et al. 2007). Godoy et al. (2006) also found evidence that MazF plays a role in killing after hydroxyurea treatment of *E. coli*, and argued that both views might be correct. Their argument is that MazF induction leads to growth inhibition that is usually reversible, but that certain extreme conditions (such as hydroxyurea treatment) put cells in a position from which they cannot recover from the induced growth stasis.

A very recent study presented evidence that a MazF-dependent PCD pathway interacts with an apoptotic-like death pathway that is SOS dependent (Erental et al. 2012). Support for a general role of MazF in PCD comes the demonstration that MazF is essential for a PCD pathway in *Myxococcus xanthus* development (Nariya and Inouye 2008). There is clearly much to be learned about the precise role of MazF and other chromosomally encoded toxins under various stressful conditions.

Hydroxyl Radical Generation and PCD

In 2007, Kohanski et al. (2007) reported that bacteriocidal antibiotics, including ones that do not induce DNA damage (e.g., aminoglycosides), induce the production of deleterious hydroxyl radicals. Multiple experiments indicated that radical production contributes to cell death after antibiotic treatment. The proposed PCD pathway involves the tricarboxylic acid cycle, transient depletion of nicotinamide adenine dinucleotide, destabilization of iron–sulfur clusters, and stimulation of the Fenton reaction. A later study provided evidence that a major cause of the PCD is induced oxidation of guanine to 8-oxo-guanine in the nucleotide pools, which, on incorporation into nucleic acids, was proposed to provoke double-strand breaks in DNA and mistranslation of mRNA (Foti et al. 2012).

Dwyer et al. (2012) reported that this proposed PCD pathway is closely related to eukaryotic apoptosis. They found hallmarks of eukaryotic-like apoptosis in *E. coli*, including

phosphatidyl serine exposure on the cell surface, chromosome condensation, and DNA fragmentation. In addition, they found that RecA binds to peptides that also serve as substrates for eukaryotic caspases, and that degradation is regulated by ClpXP protease under conditions of cell killing (Dwyer et al. 2012). An initial result of the induction of oxidative damage is RecA-dependent induction of SOS, and so the SOS response appears caught in the middle of the PCD pathway.

Two recent studies, however, have cast doubt on the role of hydroxyl radical production in PCD by failing to confirm important predictions of the model. Neither hydrogen peroxide nor intracellular-free iron were induced after antibiotic treatment as would be expected, and lethality persisted in the absence of oxygen (Keren et al. 2013; Liu and Imlay 2013).

PCD in *Caulobacter*

As mentioned above, *C. crescentus* induces an SOS response that includes a cell-cycle checkpoint. At higher levels of DNA damage and/or in the absence of a functional SOS response, this species also induces a PCD pathway that resembles eukaryotic apoptosis (Bos et al. 2012). Cell death is associated with positive staining by two apoptosis-indicative dyes, one that is dependent on membrane depolarization and the other on chromosome fragmentation. In this case, chromosome cleavage was shown to be dependent on an endonuclease christened BapE for bacterial apoptosis endonuclease, which is both necessary and sufficient for the induced cell death (Bos et al. 2012).

SOS-INDEPENDENT DNA DAMAGE RESPONSES

The SOS pathway is augmented by newly discovered DNA damage response pathways in some bacterial species, and in others, all DNA damage response appears independent of SOS. In *E. coli*, the adaptive response to DNA alkylation (see below) has been well studied for decades, and transcriptome analyses uncovered additional LexA-independent but damage-in-

ducible networks of genes that remain to be studied (Courcelle et al. 2001; Zheng et al. 2001; Khil and Camerini-Otero 2002; Friedberg et al. 2006). This section highlights a few novel pathways that augment or replace SOS in response to DNA damage.

The Adaptive Response: AidB and DNA Protection in Starved Cells

As reviewed by Friedberg et al. (2006), the adaptive response involves the transcriptional induction of several genes in response to alkylation damage to the DNA. Alkyltransferase protein Ada, on transfer of a methyl group from DNA to the Cys38 residue, becomes a transcriptional activator for genes including *ada*, *alkA*, *alkB*, and *aidB* (Fig. 3A). In addition to its role in regulation, the Ada protein facilitates DNA re-

pair directly via alkyltransferase activity. AlkA, on the other hand, is a DNA glycosylase that initiates base excision repair of alkylated bases. Recent results have advanced our understanding of the function of the AidB protein.

Almost 20 years ago, AidB was noted to be homologous to acyl-coenzyme A (acyl-CoA) dehydrogenases, and cells overexpressing the protein had both increased isovaleryl-CoA dehydrogenase activity and reduced mutagenesis after treatment with an alkylating agent (Lanini et al. 1994). Notably, based on their results, these investigators proposed that AidB might serve a detoxification role. Subsequent biochemical and structural studies supported the assignment of AidB as an acyl-CoA dehydrogenase, and also showed that AidB contains a small carboxy-terminal DNA-binding domain (Rohankhedkar et al. 2006; Bowles et al. 2008).

Figure 3. SOS-independent DNA damage responses. (A) In the adaptive response, transfer of an alkyl group from DNA to the Cys-38 residue of the Ada protein generates the activator for the adaptive response, which promotes transcription of the *ada* gene along with *alkA*, *alkB*, and *aidB* genes. (B) Most damage-inducible genes in mycobacteria are regulated by ClpR, a transcriptional activator that binds to the promoter region of its own gene as well as that of the *recA*, *clpP*, and other regulon genes. The *recA* gene is also controlled by LexA and the SOS response from the second of two *recA* promoters.

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The functional relevance of the DNA-binding domain is quite remarkable. AidB was characterized as a locus-specific detoxification protein (Rippa et al. 2011; also see Rippa et al. 2010). AidB protein binds, via the carboxy-terminal domain, to UP elements located at strong promoters, promoting efficient transcription of various genes including DNA repair and housekeeping genes. Strikingly, AidB was found to protect such downstream genes from alkylation damage. The investigators suggested that the protein, via its dehydrogenase active site, inactivates alkylating agents as they approach DNA. When the DNA-binding domain was ablated to result in free-floating AidB, it provided a general protective effect. The protective action of AidB is reminiscent of the bacterial Dps proteins (Zhao et al. 2002; Friedberg et al. 2006). Dps is present at high levels in stationary phase cells and is also induced in response to oxidative damage by the OxyR regulatory system (Altuvia et al. 1994). Like Dps, AidB is also induced in stationary phase in a σ^S -dependent fashion (Volkert et al. 1994; Lacour et al. 2002), and therefore these two proteins collaborate to protect DNA from oxidative assault during starvation.

ClpR Regulation in Mycobacteria

Although mycobacteria have an SOS system, most damage-inducible genes are instead controlled by a regulator in the ClpR family (Rv2745c in *M. tuberculosis* and Ms2694 in *Mycobacterium smegmatis*; Rand et al. 2003; Gamulin et al. 2004; Wang et al. 2011). The ClpR regulator binds to the promoter regions of many damage-inducible genes, including DNA repair genes, recognizing an altered promoter structure in which -10 and -35 consensus elements are separated by an unusually short 8 bp (Fig. 3B) (Wang et al. 2011). Interestingly, the *recA* gene itself is up-regulated from two different promoters, one in a LexA (SOS)-dependent fashion and one in a ClpR-dependent fashion. In *M. tuberculosis*, the ClpR response is induced by a broad array of stresses, including diamide, heat, SDS, hypoxia, infecting macrophages, vancomycin, low pH, and DNA damage (see Wang

et al. 2011 for citations). The ClpR family of regulators was first uncovered as transcriptional activators of Clp protease (Bellier and Mazodier 2004; Engels et al. 2004; Ventura et al. 2005). Thus, the response is more accurately classified as a broad stress response with an important component related to DNA damage and its repair. All mycobacteria and some other actinomycetes carry conserved homologs of Rv2745c, suggesting that the ClpR response pathway is broadly spread in this group of bacteria.

Damage Response in the Highly Radioresistant *Deinococcus radiodurans*

D. radiodurans has the remarkable ability to reconstitute its genome after very high levels of double-strand breaks and other forms of DNA damage induced by ionizing radiation (IR) or desiccation, which is likely the natural stress that led to the evolution of its radioresistance (for reviews, see Mattimore and Battista 1996; Battista 1997; Cox et al. 2010; Slade and Radman 2011). The high level of resistance depends on both efficient DNA repair pathways and an unusually robust antioxidation system that protects proteins, including critical repair proteins, from oxidative damage and inactivation (Daly et al. 2007, 2010; Daly 2009; see “Reconstitution of Heavily Damaged Chromosomes” below for discussion of DNA repair mechanisms). As expected, resistance to IR also depends on robust regulation of repair and protective proteins. Using microarray expression approaches, Tanaka et al. (2004) identified >70 IR-induced genes in this organism, including both DNA repair proteins (e.g., RecA) and proteins involved in alleviating oxidative stress.

The *recA* gene of *D. radiodurans* is induced after DNA damage, but surprisingly, neither of the two LexA homologs of the organism is required for activation although they are cleaved in a RecA-dependent manner (Narumi et al. 2001; Bonacossa de Almeida et al. 2002; Earl et al. 2002; Hua et al. 2003; Satoh et al. 2006; Slade and Radman 2011). The *recA* gene and at least 28 additional genes, many encoding DNA repair proteins, contain a conserved motif called the radiation/desiccation response motif

(Makarova et al. 2007). Recently, a key regulatory protein, PprI (also called IrrE and DR0167), was shown to bind the *recA* (and *pprA*) gene promoters in vitro, and interestingly, was found at the IR-inducible promoters in vivo only after DNA damage (measured by ChIP; Lu et al. 2012). PprI itself is constitutively expressed, suggesting that DNA damage leads to posttranslational activation of PprI by some mechanism, but the details are currently unknown.

In addition to the key role of PprI, several protein kinases and related proteins have been implicated in the response to DNA damage in *D. radiodurans*: DrRRA (DR2418), an apparent response regulator of a two-component-like system (Wang et al. 2008); DR2518, a predicted transmembrane protein of the serine-threonine kinase family (Rajpurohit and Misra 2010); and RadRS (DRB0091/DRB0090), a two-component system that includes the RadS histidine kinase, which can phosphorylate both itself and its partner RadR, a DNA binding protein with currently unknown targets (Desai et al. 2011). Further investigations are needed to understand the precise and apparently overlapping roles of PprI, DrRRA, DR2518, and the RadRS proteins in the DNA damage response in *D. radiodurans*, and to explore whether damage-inducible protein kinases are used broadly in bacteria.

DNA Damage Induction of Competence Proteins

In some species, DNA damage induces competence proteins rather than proteins involved in DNA repair (for review, see Charpentier et al. 2012). Such is the case for both *Streptococcus* species and *Helicobacter pylori*, both of which lack any obvious LexA homolog (Prudhomme et al. 2006; Dorer et al. 2010, 2011). Despite the lack of LexA in *H. pylori*, RecA is still required for induction of competence (and other) proteins (Dorer et al. 2010). Amazingly, competence itself is also required for the up-regulation seen after DNA damage, leading to a positive feedback loop (Dorer et al. 2011). The mechanism of this remarkable regulatory loop is unclear, but it has been suggested that either up-

take of exogenous DNA is needed for the inducing RecA-DNA complexes, or that RecA associates with the competence machinery to mediate transcriptional signaling (Dorer et al. 2011). The significance of the connection between competence and DNA damage demands further study; presumably, transformation speeds the generation of novel variants during stressful conditions and perhaps imported exogenous DNA provides a template for chromosomal recombinational repair (Dorer et al. 2011).

PROKARYOTIC STRATEGIES FOR REPLICATING DAMAGED DNA

Bacterial replication machineries and the proteins that underpin replication (mostly recombination proteins) allow dramatic and powerful strategies for completing the replication of damaged DNA. There is now evidence that replication forks can jump forward (reprime the leading strand), back up (regress), and repair after getting broken, perhaps even undergoing intentional breakage followed by repair. In addition, traditional repair can be coupled to the process of replication, either when fork regression regenerates a repair template in the parental DNA (see below) or by means of a coupled recombination-excision repair event after the fork passes (Bichari et al. 2009). A major challenge for the field is to decipher the interplay between these various pathways and how the appropriate pathway is chosen for a particular form of DNA damage.

An overview of some of these topics with recent advances will be presented here, and the reader is referred to comprehensive reviews of various aspects that have appeared in recent years (Cox et al. 2000; Michel 2000; Cox 2001; Lusetti and Cox 2002; McGlynn and Lloyd 2002; Courcelle and Hanawalt 2003; Kreuzer 2005; Friedberg et al. 2006; Heller and Marians, 2006b; Michel et al. 2007; Atkinson and McGlynn 2009; Gabbai and Marians 2010). The reader may also be interested in the related topic of replication-transcription collisions discussed in recent reviews (Pomerantz and O'Donnell 2010; McGlynn et al. 2012).

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Repriming of the Leading Strand Ahead of the Fork

In contrast to simple textbook models in which the leading strand of a bacterial replication fork is synthesized continuously whereas the lagging strand is discontinuous, analyses of *in vivo* replication have suggested discontinuities in both newly synthesized strands, particularly after DNA damage (for discussion of early studies, see Friedberg et al. 2006; also see Amado and Kuzminov 2006). How could leading-strand replication reinitiate after a blocking lesion stalls the leading-strand DNA polymerase (Fig. 4A)? One model invokes the use of RNA polymerase transcripts hybridizing to the parental leading-strand template downstream of the blocking lesion (Camps and Loeb 2005; Friedberg et al. 2006). Recent biochemical studies have uncovered a second attractive model. Heller and Marians (2006a) showed that under appropriate conditions, the DnaG helicase of the *E. coli* replisome can synthesize primers to allow reinitiation of leading-strand synthesis downstream of a block. In a later study, this pathway was shown to operate without any dissociation and reloading of the replication machinery, demonstrating an inherent tolerance of the replisome to blocking lesions (Yeeles and Marians 2011).

Replication Fork Breakage and Restart

As discussed in detail by Friedberg et al. (2006), homologous recombination provides an excellent pathway to restart broken replication forks (also see Kuzminov 1999; Cox et al. 2000; Kreuzer 2005; Michel et al. 2007). Replication forks can become broken by encounters with template nicks (Kuzminov 2001) and from fork cleavage reactions such as those mentioned in “Fork Regression and Template Switching” below. The basic pathway for reconstituting broken replication forks involves a one-ended strand invasion reaction in which the broken end invades the intact homologous duplex from which it had broken away (Fig. 4B). After D-loop formation, the invading 3' end is used as a primer for synthesis of a new leading strand

and the displaced single-stranded region is a loading site for a helicase–primase complex, allowing lagging-strand synthesis (Fig. 4B). This pathway has been reconstituted *in vitro*, and depends critically on the PriA protein for the loading of replicative helicase DnaB onto the D-loop intermediate (Liu and Marians 1999; Liu et al. 1999; Xu and Marians 2003).

Fork Regression and Template Switching

Because the two daughter strands have complementary sequence, they are able to form a duplex with each other if unwound from parental template DNA in a reaction called replication fork regression (Fig. 5). The possibility that regression could serve as a damage bypass mechanism was recognized decades ago (Fujiwara and Tatsumi 1976; Higgins et al. 1976).

Replication fork regression can be considered as either productive or pathological, and it seems likely that this balance is determined by the nature of the blocking agent and the available repair/recombination proteins (Atkinson and McGlynn 2009). The potentially productive reactions that can be promoted by regression include clearing the parental template DNA for a repair reaction, allowing template switching that can bypass a template lesion, and reinvagination of the extruded arm into the template duplex ahead of the (regressed) fork (Fig. 5A, B, and C, respectively). An excellent and comprehensive review on fork regression was published recently (Atkinson and McGlynn 2009), and only a brief overview will be presented here.

Evidence that stalled or blocked *E. coli* chromosomal replication forks that undergo regression first arose when Michel and colleagues found double-strand chromosomal breaks when the cells carried debilitating mutations in certain replication proteins (Michel et al. 1997). Detection of the breaks required inactivation of RecBCD, presumably due to a major role of RecBCD in processing the extruded arm at the regressed fork. The subsequent finding that RuvABC is required for break formation argued that fork regression, which creates a Holliday junction at the fork, was involved in the chromosomal breakage pathway (Seigneur et al.

Figure 4. Models of fork rescue via repriming of the leading strand and a recombinational repair reaction. (A) A blocking lesion on the leading-strand template can be overcome by a new priming event downstream of the blocking lesion. As described in the text, a nascent RNA synthesized by RNA polymerase could provide a primer (green), as could the DnaG primase in the replisome. All newly synthesized DNA is in red, and the blocking lesion is indicated by the small lightning bolt. (B) A broken replication fork can be reconstituted by homologous recombination. In this model, the broken end undergoes strand invasion to create a D-loop structure, which serves as assembly site for a new rightward facing replisome. Branch migration and Holliday junction resolution can remove the branched connection between the two daughter molecules. All newly synthesized DNA is in red, and the blue arrows indicate one of several possible junction resolution reactions (cleavage of the cross strands followed by religation). pol, Polymerase.

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Figure 5. Models of fork rescue via fork regression. (A) When the replication fork encounters a template lesion (small lightning bolt), fork regression can return the lesion into an environment of simple duplex DNA to facilitate a traditional repair event such as excision repair. (B) In cases in which lagging-strand synthesis extends beyond the site of a lesion on the leading-strand template, fork regression provides an opportunity for a strand switching event in which polymerase extension on the extruded arm (DNA in green) effectively bypasses the lesion so that replication can be completed. This gives the cell another cell division cycle to repair the damage using conventional repair pathways. Fork regression can be resolved either by reversal of fork regression (A,B), nucleolytic digestion of the extruded arm (not shown), or (C) strand invasion of the extruded arm ahead of the replication fork. In this figure, all newly synthesized DNA is in red (except for the green template switch patch).

1998). Physical evidence consistent with replication fork regression in *E. coli* was later obtained using neutral–neutral two-dimensional agarose gel electrophoresis of replicating damaged plasmids (Courcelle et al. 2003), although the detected intermediates might possibly have been some other branched form that migrated in a similar region of the gel. An analysis in phage T4-infected *E. coli* provided more definitive physical evidence of *in vivo* fork regression by characterizing site-specific regressed forks (Long and Kreuzer 2008, 2009). Replication fork regression can be driven, at least *in vitro*, by positive supercoiling of the template ahead of the fork, any of a number of helicases that act on branched DNA, and by strand exchange proteins (for review, see Atkinson and McGlynn 2009).

Khan and Kuzminov (2012) carefully analyzed replication and repair after UV treatment in *E. coli*. They reached the surprising conclu-

sion that fork regression, followed by induced breakage of the regressed fork, was necessary for survival under certain conditions of DNA damage. The breaks were not detected in wild-type cells because of efficient break repair involving RecA and RecBCD. Evidence that the induced breakage pathway is important for survival comes from the requirement for both RecA and RuvABC for optimal survival under these conditions (Khan and Kuzminov 2012). The investigators argue that this pathway becomes critical when DNA damage is closely spaced on the two template strands, but not so closely spaced that regression is impossible. Induced cleavage in this situation is proposed to give the parental template more time to repair via the nucleotide excision repair pathway before the recombination events that restart the broken forks (Khan and Kuzminov 2012). Further experiments are needed to understand the balance between this pathway of induced breakage and

the arguably simpler pathway of temporary regression to expose template damage to the repair machinery (as in Fig. 5A).

Reconstitution of Heavily Damaged Chromosomes

As mentioned above in “ClpR Regulation in Mycobacteria,” *D. radiodurans* has the remarkable ability to reconstitute its genome after very high levels of double-strand breaks and other forms of DNA damage (for review, see Battista 1997; Cox et al. 2010; Slade and Radman 2011). This genome reconstitution was shown to involve two major phases, the first relying on a special form of DNA replication (Zahradka et al. 2006). The so-called extended synthesis-dependent strand-annealing pathway in the first phase begins with extensive exonucleolytic digestion to generate invasive 3' ends. The heart of this first phase is then a replication pathway that extends the 3' ends of the numerous DNA fragments, using unbroken regions of homologous template strands. As in transcription, the newly synthesized strands are somehow dissociated from the template. This generates many 3' extensions, and in the second stage, annealing of complementary extensions begins to piece together longer stretches of the genome in a RecA-dependent process, culminating in completion of an intact duplex genome (Zahradka et al. 2006). More recent studies have elucidated detailed protein requirements and mechanistic steps in the genome reconstitution pathway (Slade et al. 2009; Bentschikou et al. 2010). Remarkably, selection for radioresistant *E. coli* successfully generated strains that are nearly as resistant as *D. radiodurans*, revealing that a relatively small number of mutational changes can allow *E. coli* to follow a similar (or at least an effective) pathway of genome reconstitution (Harris et al. 2009).

CLOSING REMARKS

The field of DNA damage responses in bacteria continues to provide extremely interesting biology and mechanism. Transcriptional responses including SOS regulate not only DNA repair

enzymes, but also very diverse proteins involved in cell division, genetic rearrangements and exchange, mutation, and virulence factor production. Furthermore, DNA damage responses are variable between bacterial species, apparently being adapted for particular needs and ecological lifestyles. Survival after DNA damage is influenced by dynamic regulatory mechanisms as well as cell growth inhibition, death pathways, and impressive replication fork gymnastics in response to template lesions. Future studies should provide a much better understanding of how these multiple layers complement each other for optimal survival.

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