Upstream of the SOS response: figure out the trigger

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The bacterial SOS regulon encodes a response to DNA damage that not only functions to relieve the incurred damage but also enhances adaptation through mutagenesis and the lateral spread of virulence factors. Recent papers have demonstrated that certain stimuli can indirectly generate the SOS-inducing signal by activation of endogenous DNA damage mechanisms rather than by direct DNA damage. We suggest that these endogenous triggers have been recruited by bacteria to enable adaptation to various types of stresses.

SOS induction from within

The SOS regulon has typically been studied in relation to DNA damage caused by irradiation or chemicals. It encodes an elaborate reaction that extends beyond DNA repair and influences many other cellular processes (Box 1). This observation feeds speculations that the SOS response might not be solely dedicated to cellular defense against obvious DNA damaging treatments but, rather, could have a broader physiological function. An eminent question that follows is whether conditions other than direct DNA damage lead to SOS activation, and how this activation is triggered in the cell.

The first indication that the SOS response can be induced from within the cell was provided some time ago with the discovery that aging colonies of *Escherichia coli* display a marked increase in SOS induction [1]. Surprisingly, this phenomenon was shown to rely on the *cya* gene, which is responsible for intracellular cAMP levels, and indicates the dependence of this phenomenon on cellular metabolism. More recently, it was shown that in the absence of RecBC activity, mutants in *pta* or *ackA* were non-viable, presumably because of accumulation of DNA double-strand breaks (DSBs) [2]. *Pta* and *AckA* have opposite effects on the intracellular amounts of acetyl phosphate originating either from acetate or acetyl coenzyme A, which suggests an effect of an unbalanced acetyl phosphate pool on DNA integrity. Interestingly, the *pta* *recBC* lethality could be avoided by the inactivation of several yet uncharacterized genes, and this approach could identify the key trigger that relays information about the metabolic status of the cell into a change in DNA integrity that leads to SOS activation.

Although this exact cellular link to DNA damage and RecA activation remains to be identified, these studies show the existence of novel and endogenous pathways

for SOS induction that are intricately embedded in the general physiology of the cell.

Do stress-induced pathways upstream of the SOS response exist?

If such novel SOS triggers were found to be responsive to stress conditions, they could constitute dedicated pathways that transmit a certain stimulus (unrelated to DNA damage) to the SOS regulon, thereby endowing the cell with the possible benefits of SOS behavior when needed.

The CcdAB pathway

One such dedicated pathway in which cellular players can readily be identified could be the *ccd* system, which was first recognized as one of the weaker plasmid-addiction modules present on the F plasmid of *E. coli*. It encodes the CcdB protein, which poisons *E. coli* DNA gyrase in a similar way to the chemically synthesized quinolone antibiotics (such as nalidixic acid). In the process of transiently introducing a DSB to relieve topological constraints in DNA, the transitory cleaved DNA-gyrase complex is trapped by the CcdB protein, which prevents resealing of the DSB and triggers SOS induction. However, the *ccd* operon also encodes an antidote, CcdA, which titrates the CcdB toxin away from its gyrase target [3]. As is typical for toxin-antitoxin tandems, CcdA is an unstable protein that is rapidly degraded by the Lon protease, which contributes to the post-segregational killing phenomenon of F-plasmid-free daughter cells. Moreover, additional *E. coli* proteins such as GroE, PmbA (TldE), TldD and the carbon-storage regulator CsrA were shown to affect CcdB toxicity [3]. Some of these proteins are responsive to environmental cues and it can therefore be anticipated that, even in plasmid-containing cells, CcdB liberation and concomitant SOS induction can be modulated by stresses that elevate Lon levels or compromise CcdA synthesis, for example. Interestingly, recent experimental evidence on the effects of starvation on *ccd* expression and the CcdA:CcdB ratio has revealed the possibility of CcdB-mediated mutagenesis in resting cells [4].

The DpiBA pathway

A more intricate pathway upstream of the SOS regulon with a specific trigger was unexpectedly revealed by β -lactam-induced cell wall stress. When cell wall integrity is affected by compromising the activity of penicillin-binding protein 3 (encoded by *ftsI*), either chemically (by exposure to some β -lactams) or genetically (by introducing a temperature-sensitive *ftsI* allele), the DpiBA

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Box 1. The SOS response and its behavior: more than just repair

The bacterial SOS response is a beautifully orchestrated response to DNA damage and is triggered by the sensing of long-lived single-stranded DNA (ssDNA). Depending on the nature of the damage, either the RecBCD or RecFOR complex presents this ssDNA to the RecA protein, which subsequently forms a nucleoprotein filament that forces the protein into an activated state. Activated RecA then stimulates autocleavage of the LexA repressor protein, thereby derepressing at least 30 genes that constitute the SOS regulon [15,16].

The expressed SOS functions not only repair the DNA damage and restore the replication fork but also drive mutagenesis and the lateral spread of mobile genetic elements. These promiscuous elements often carry virulence or antibiotic-resistance genes and range from prophage to the more recently discovered integrative and conjugative elements [17]. The SOS response increases the available genetic repertoire and disseminates pathogenic traits, thereby enhancing survival and contributing to bacterial evolution. However, it should be emphasized that SOS induction also causes an immediate interference with cellular homeostasis and can delay cell division or even control respiration [18]. Intriguingly, it was recently discovered that stalled replication forks could eventually trigger programmed cell-death mechanisms and cell lysis, executed by MazEF and RelBE toxin-antitoxin systems [19], which indicates a profound connection between the SOS regulon and cellular physiology.

two-component signal transduction system becomes activated [5]. When induced, the DpiA effector protein binds to A+T-rich sequences in the replication origins of the *E. coli* chromosome. As a result, DpiA competes with the binding of DnaA and DnaB, thereby interrupting DNA replication and inducing the SOS response [6].

An immediate advantage of this induction resides in the fact that Sula (SfiA), a member of the SOS regulon, prevents cell division by inhibiting FtsZ ring formation. This consequent delay in cell division provides temporary protection from β -lactam lethality [5]. In the long term, development of resistance against sublethal exposure to the cell wall stressor could be favored by SOS-mediated mutagenesis, and it was indeed shown that error-prone DNA polymerase Pol IV (DinB) activity, which is part of the SOS regulon, is also induced by β -lactam antibiotics [7]. Interestingly, some DinB induction still occurred in the absence of a functional SOS response, which perhaps indicates that even shunt pathways to the SOS response might be cut short to obtain targeted induction of the desired SOS function. This might be particularly common for *dinB* expression, which also tends to be induced by starvation and the heat-shock response [8,9]. A similar connection between cell wall stress and SOS induction has also been reported in *Staphylococcus aureus* cells that are exposed to β -lactam antibiotics [10].

The Mrr pathway

Another recent example of a surprising environmental SOS trigger in *E. coli* is high hydrostatic pressure (HP), a physical stress [11]. Because RecA activation in this case depends on RecB, the SOS signal generated by HP could be assumed to be a DSB. However, thermodynamic constraints preclude covalent bond breaking as a direct consequence of HP, and a subsequent screen revealed that the endogenous Mrr restriction endonuclease of *E. coli* is

responsible for the actual generation of DSBs after HP stress [12]. Mrr is a cryptic member of the type IV restriction endonucleases, which are characterized by their peculiar specificity for methylated DNA. Until now, Mrr was believed only to restrict incoming DNA with unusual methylation patterns, thereby controlling the immigration of foreign genetic material. How the perception of HP stress is relayed to Mrr activation remains unknown; however, it is the first report of an endogenous restriction endonuclease that actually targets its own genome under stress conditions. Indeed, in times of stress, endogenous restriction activity tends to be carefully diverted from the chromosome by elegant restriction alleviation mechanisms. One of these alleviation mechanisms interferes with the HsdRMS system (which constitutes the type IA restriction-modification enzyme complex EcoKI) and involves ClpXP-dependent proteolysis of HsdR only when it is about to act on self-DNA [13]. Although the *mrr* gene is located next to the *hsdRMS* locus, its activation instead of alleviation seems to suggest another role in stress physiology.

Interestingly, recent work underscored the importance of DSB repair in stress-induced adaptive mutagenesis, gene amplification and chromosomal rearrangements. In the model system of starvation-induced reversion of a *lac* mutation located on the F' conjugative plasmid, it seems that the F'-encoded TraI endonuclease is responsible for the generation of DSBs [14]. In this light, it might not be surprising that resident endonucleases or gyrase poisons are accustomed to gently targeting their own chromosome in times of stress to elicit such adaptation mechanisms.

Concluding remarks

The emerging role of the SOS response in general bacterial stress adaptation urges for an adaptation of the traditional SOS response paradigm itself. Several environmental or intracellular signals seem to be relayed by specific proteins to endogenous DNA-damaging mechanisms, turning on the SOS response and its inherent adaptive potential. Although at present it cannot be excluded that these indirect SOS triggers are the trivial result of a perturbation of normal cell function, the observation that they are intricately embedded in cellular physiology and have recruited specific proteins for signal transduction suggests that they have been rewarded by evolution based on their selective advantage.

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Genome Analysis

Thermophiles like hot T

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A plethora of mechanisms confer protein stability in thermophilic microorganisms and, recently, it was suggested that these mechanisms might be divided along evolutionary lines. Here, a multi-genome comparison shows that there is a statistically significant increase in the proportion of NTN codons correlated with increasing optimal growth temperature for both Bacteria and Archaea. NTN encodes exclusively non-polar, hydrophobic amino acids and indicates a common underlying use of hydrophobicity for stabilizing proteins in Bacteria and Archaea that transcends evolutionary origins. However, some microorganisms do not follow this trend, suggesting that alternate mechanisms (e.g. intracellular electrolytes) might be used for protein stabilization. These studies highlight the usefulness of large-scale comparative genomics to uncover novel relationships that are not immediately obvious from protein structure studies alone.

Protein thermostability: a mechanistic distinction between Bacteria and Archaea?

Protein structure information has revealed an extensive repertoire of mechanisms that help to maintain protein integrity and function at high temperatures [1]. Thermophilic proteins can exhibit higher core hydrophobicity [2],

greater numbers of ionic interactions [3], increased packing density [4], additional networks of hydrogen bonds [5], decreased lengths of surface loops [6], stabilization by heat-stable chaperones [7], an increase in disulfide bond formation [8] and a general shortening of length [9]. Changes in amino acid composition that account for some of these mechanisms can be detected in proteome-wide surveys. These include elevated levels of lysine, valine and glutamic acid, arginine-to-lysine replacement and a decrease in glutamine and histidine in thermophiles that cannot be explained by genomic G+C compositional biases or by universal trends of amino acid gain and loss in protein evolution [9–13].

Recently, it was suggested that mechanisms for conferring protein stability could be divided along evolutionary lines [14]. Thermophilic Archaea, as exemplified by *Pyrococcus furiosus*, are postulated to have evolved in hot places and to use a structure-based method for protein stability: proteins of high density that result from the contribution of numerous mechanisms including higher core hydrophobicity and tighter atom packing. By contrast, thermophilic Bacteria such as *Thermotoga maritima* are thought to have entered hot environments after evolving elsewhere. It has been suggested that they employ a sequence-based method of protein stability in which protein density is similar to that of mesophiles but with strategically placed salt-bridges to confer thermostability. Here, we suggest that at least one mechanism for protein

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