Transcriptional responses to DNA damage

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In *Escherichia coli*, DNA repair and protective responses are regulated at the transcriptional level. Regulatory mechanisms have evolved that allow cells to respond to DNA damage by mounting the appropriate responses. The regulatory proteins controlling these responses are activated when they recognize the presence of a specific DNA damaging agent, the production of specific DNA lesions, or the production of damage intermediates resulting from replication of lesions containing DNA. Transcription of the responses to DNA damage are induced when the activated regulatory proteins stimulate transcription of the genes they control by a variety of complex and unique molecular mechanisms.

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Abbreviations

BER	base excision repair
^{me}Ada	methylated Ada
NER	nucleotide excision repair
RNAP	RNA polymerase
ROS	reactive oxygen species
0^4meT	0^4methylthymine
0^6meG	0^6methylguanine

Introduction

The prevention of the lethal and mutagenic consequences of DNA damage requires the timely expression of DNA repair genes and protective genes in order to maintain the integrity of the genome and viability of the cell. When replication of damaged DNA occurs prior to repair, two different events can occur. Lesions such as 0^6methylguanine (0^6meG), 8-oxoguanine (8-oxoG) and 0^4methylthymine (0^4meT) can be misreplicated, resulting in mutations [1,2]. Lesions such as pyrimidine dimers, N^3methyladeneine, and thymine glycols can block replication, leading to cell death [3–6]. To prevent these consequences, cells have evolved mechanisms that repair or prevent DNA damage.

To regulate DNA repair responses, *E. coli* has evolved sensors and regulatory circuits that recognize the presence of a DNA damaging agent or the consequences of its action on DNA and induce the appropriate DNA repair or protective response(s). The regulatory mechanisms involved in the control of transcription of DNA repair genes are complex and provide interesting and unique insights into transcriptional regulatory mechanisms.

A general regulatory scheme, outlined by Neidhardt [7], to describe regulation of stress responses can be used to explain the transcriptional responses to DNA damage (Table 1). As shown in Table 1, cells can respond to two kinds of signals: the presence of a DNA-damaging agent, or DNA damage. Either signal can activate specific regulatory genes that, in turn, induce the repair response. There are several mechanisms by which DNA repair response regulators are activated to trigger induction of the responses they control. The regulatory proteins can be directly activated by the DNA damaging agent (SoxR and OxyR), by the DNA damage (RecA), or as a result of its own DNA repair activity (Ada). Whereas the SoxR, OxyR and Ada regulatory proteins act as transcription activators, RecA protein triggers derepression of the SOS regulon by inactivation of a repressor protein.

Regulation of the SOS response

The most extensively studied transcriptional response to DNA damage is the SOS response (see reviews [3,8]). This involves at least 31 genes [9••] and includes genes required for several general DNA repair mechanisms, such as nucleotide excision repair (NER) (Figure 1a), recombinational repair (Figure 2a), and new polymerases involved in mutagenic bypass replication of lesions [10••,11] (Figure 2b). NER can repair essentially any bulky lesion by cutting out a 12-base single-stranded region containing the lesion, but fails to repair, or repairs very poorly, small lesions such as methylated and oxidized bases [12–15]. Recombinational repair is required for repair of strand breaks and gaps, and mutagenic translesion synthesis by polymerase V of *E. coli* is required to replicate past lesions in single-stranded template regions [11]. Most DNA-damaging agents, even those that produce lesions not efficiently repaired by NER, cause induction of the SOS response and require *recA*-dependent recombinational repair to assist in recovery from damage [15–18]. Consistent with its general DNA repair functions, the SOS system has evolved to respond to a general signal: single-stranded DNA produced by most DNA-damaging agents. Single-stranded DNA can be produced by a variety of mechanisms [19-21]. First, single-stranded DNA breaks can be converted to single-stranded gaps by exonucleolytic digestion of the broken strand [22,23]. Second, double-stranded DNA breaks can be converted to single-stranded regions by the activity of exonuclease V, which degrades both DNA strands beginning at the break and continues until it encounters a sequence known as a chi site (5′GCTGGTGG 3′). At the chi site, digestion of the 3′ end ceases and exonuclease V continues to degrade only the 5′ end, leaving a 3′ single-stranded tail, which is a substrate for recombinational repair [24•,25]. Third, single-stranded DNA can be produced by replication of lesion-containing templates. Single-stranded parental

Table 1

Genetic responses to DNA damage and DNA damaging agents.

Stimulus	Signal	Sensor	Regulatory protein	Key regulatory reaction	Gene induction	Response
UV damage to DNA	Single-stranded DNA	RecA	RecA*	RecA* triggers autolytic cleavage of the LexA repressor, resulting in derepression of SOS genes	31 known SOS genes including *umuDC*, *uvrAB*, genes for excision repair and recombination genes, and others.	SOS response
Methylation damage to DNA	Methylphosphotriesters	Ada	^{me}Ada	^{me}Ada activates expression of all adaptive response genes.	Alkylation repair and protection genes: *ada/alkB operon*, *alkA* and *aidB*	Adaptive response to alkylation damage
Superoxide	Superoxide and nitric oxide	SoxRS	Oxidized SoxR	SoxR stimulates transcription of soxS, which induces superoxide-inducible genes.	Superoxide resistance genes: *sodA*, *micF*, *zwf* and *fumC*	Superoxide resistance response
Peroxide	Peroxide	OxyR	Oxidized OxyR	Oxidized oxyR activates transcription of peroxide protection genes	Peroxide resistance genes: *katG*, *ahpC*, *dps*, *grx* and *gorA*	OxyR response

DNA regions opposite the daughter-strand gaps are produced by DNA polymerase whenever it encounters a replication-blocking lesion in the DNA template [26]. Single-stranded DNA produced by any or all of these mechanisms can be bound by RecA protein, resulting in conversion to its activated form, called RecA*. Though the exact nature of RecA* is not yet known, once activated, RecA* must interact with the LexA protein, the repressor of the SOS genes. This RecA*–LexA interaction triggers an autolytic activity of LexA that causes its cleavage and the destruction of its ability to function as a repressor, which, in turn, results in derepression of SOS genes [27,28]. All SOS genes have sufficiently strong promoters to allow factor-independent transcription once derepressed, resulting in the induction of at least 31 genes in *E. coli* and expression of the SOS response [8,9]. The SOS induction process is identical to the induction of λ prophage. λ repressor is similar in structure and function to the LexA repressor and UV treatment of host cells carrying λ prophage results in RecA*-dependent autolytic cleavage of λ repressor [28–30].

Repair of oxidative and alkylation damage

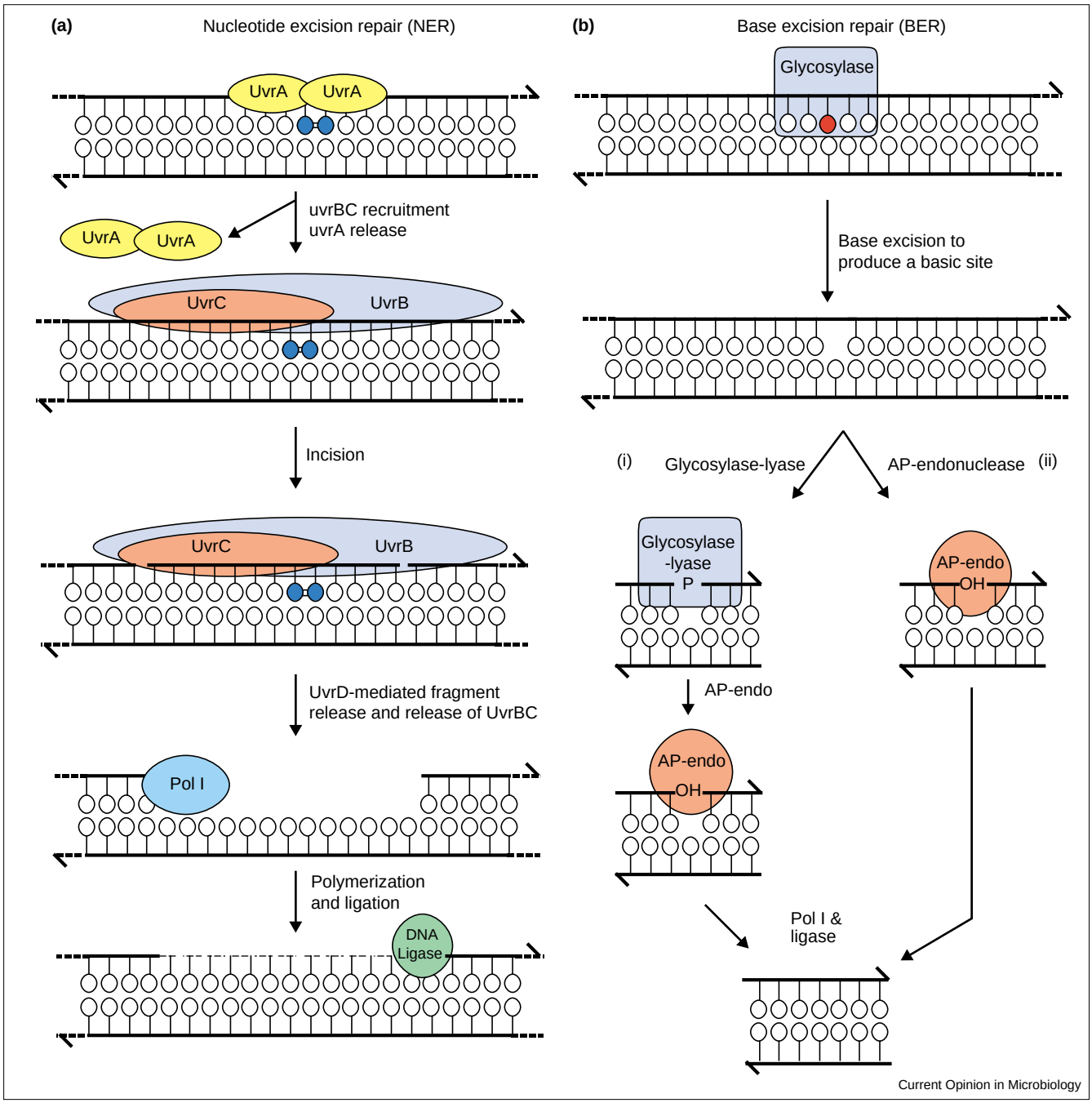
Agents that produce methyl or oxidative damage induce not only the SOS response but also elicit other more specific DNA repair responses [31–35]. Because DNA lesions produced by methylating and oxidizing agents are repaired inefficiently, if at all, by NER, additional DNA repair and protective mechanisms have evolved that are capable of repairing these types of lesions. Some of these mechanisms are expressed constitutively, whereas others respond to the appropriate type of damage and are induced. Lesions produced by methylating and oxidizing agents are typically repaired by base excision repair (BER) mechanisms (Figure 1b). The key step of this type of repair is the excision of a damaged base by a glycosylase, which acts by cleaving the glycosylic bond, thereby removing the damaged base from the sugar phosphate backbone. This leaves an abasic site, which is subsequently converted to a gap by AP endonucleases, or, in some cases, by an intrinsic lyase activity of the glycosylase itself. Filling of the gap by polymerase I and resealing by DNA ligase complete the repair [32,33,36].

Regulation of the adaptive response to alkylation damage

The adaptive response to alkylation damage is a positively-regulated response that is specifically induced by methylation damage to DNA [37]. The adaptive response genes include: *ada*, *aidB*, *alkA*, and *alkB* genes. The *ada* gene encodes the Ada protein, which has the dual function of transcriptional activator for the adaptive response and a methyltransferase that demethylates two methylated bases (O^6meG and O^4meT) and methylphosphotriesters produced by methylating agents in the sugar phosphate backbone [32,33]; the *alkA* gene, which encodes a glycosylase that repairs several different methylated bases; the *alkB* gene, which forms a small operon with *ada* and is required for error-free replication of methylated single-stranded DNA [38]; and the *aidB* gene, whose product appears to detoxify nitrosoguanines, reducing the level of methylation by these agents [39]. In addition to induction by exposure to methylating agents, these adaptive response genes are also partially induced upon entry into stationary phase; this form of induction requires *rpoS*, a gene encoding an alternative σ factor mainly active in stationary phase [40,41].

The signal that triggers the induction of the adaptive response is a methylphosphotriester. The repair of this lesion by Ada protein converts Ada to methylated Ada (^{me}Ada), a form that is active in transcription [42,43]. Thus, activation of Ada is a consequence of one of its DNA repair activities. The specificity for methylphosphotriesters as the signal is a result of the fact that Ada protein has two independent methyltransferase activities that can repair

Figure 1

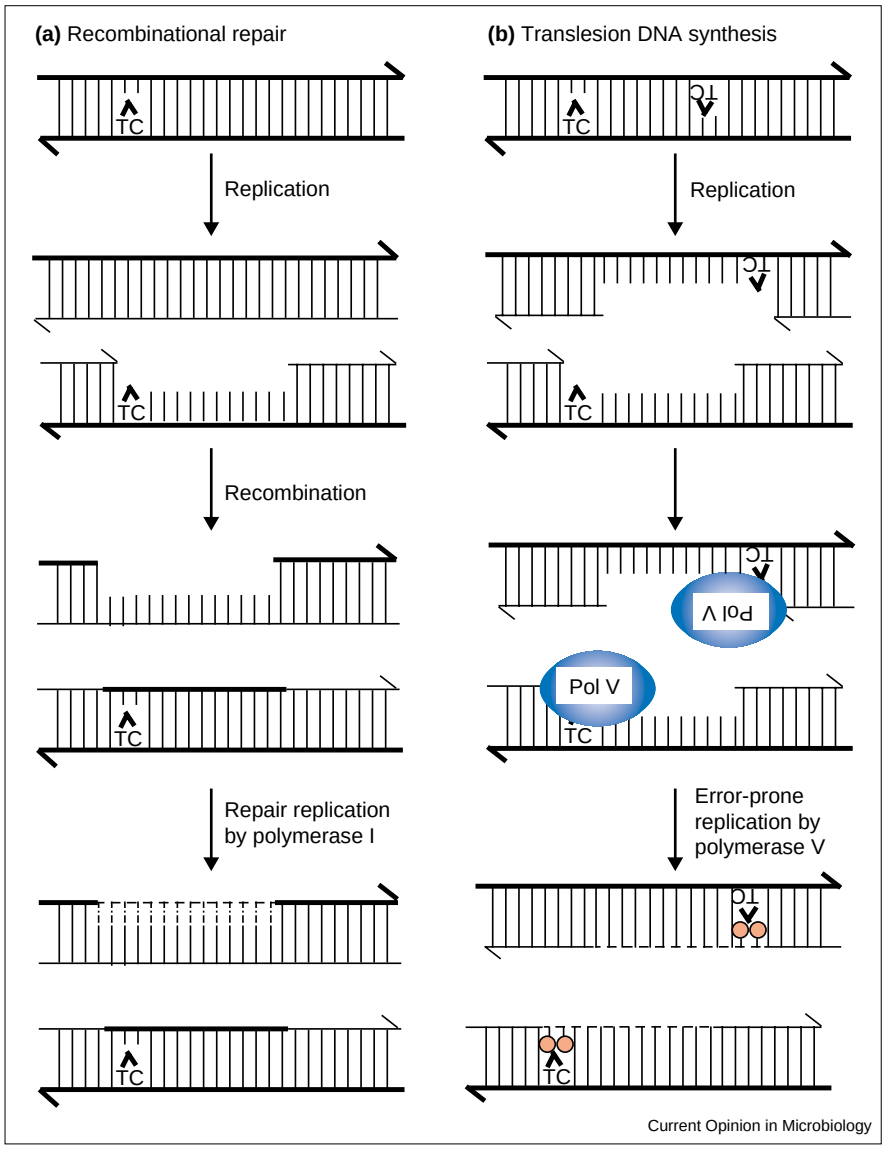


Excision repair mechanisms. (a) Nucleotide excision repair (NER) is a key DNA repair function of the SOS response. First, a dimer of UvrA binds to the lesion (blue bases), then recruits the uvrBC subunits, which cleave the DNA at two sites asymmetric to the lesion. Pol I then resynthesizes a small patch of DNA backbone to replace the damaged fragment, which is released with the assistance of UvrD helicase. DNA ligase then completes the repair process. (b) Base excision repair (BER) begins when glycosylase binds to the lesion and removes the damaged base, leaving an abasic site. (i) One of several possible outcomes of the action of glycosylase-lyase enzymes, which cleave the DNA backbone by β and/or δ elimination, leaving a variety of 3′ residues, is shown. The 3′ phosphate shown is only one of several products. The *E. coli* and yeast AP-endonucleases are able to remove 3′ phosphates as well as several other types of non-priming 3′ ends, converting them to 3′ OH groups. (ii) The repair intermediates of glycosylated lacking lyase activity are a basic sites that are recognized by AP-endonucleases that cleave the sugar phosphate backbone to produce a 3′ OH. Finally, Pol I synthesizes a small patch of backbone, and DNA ligase completes the repair process.

DNA — one contained in its amino-terminal domain and a second in its carboxyl-terminal domain [42,44]. The carboxyl-terminal domain of Ada removes methyl groups from 0^6meG and 0^4meT and transfers them to its own Cys321 residue, restoring the bases to their undamaged state [42,43]. Once Ada has removed the methyl group

Figure 2

Processing of daughter strand gaps in replicated damaged DNA. Parental DNA is shown as a thick line, daughter strands are shown as thin lines and the DNA repair replication patches are shown as dashed lines. The lesion shown is a TC pyrimidine dimer. Replication produces a daughter strand gap opposite the template lesion.
(a) Recombination with the parental strand of one molecule can fill the daughter strand gap opposite the lesion. This produces a gap in the other daughter molecule. However, its complement has an intact template that can direct resynthesis of a patch, completing the replication of two intact molecules.
(b) Translesion synthesis can allow filling of the daughter strand gap in regions that cannot be repaired by recombination. One example of such a substrate is shown. Two lesions in close proximity result in overlapping daughter strand gaps. Replication by Pol V can bypass the noncoding template lesions, resulting in the insertion of untemplated bases, which may be mutagenic (red residues).



from the base and transferred it to itself, repair is complete. The methyltransferase activity present in the amino-terminal domain of Ada is required for the removal of methyl groups from the phosphate oxygens, repairing only the Sp diastereoisomer and leaving the Rp diastereoisomer to remain in DNA, where it has no apparent deleterious effect [5,45–47]. This demethylation reaction results in the irreversible methylation of the Cys69 residue of Ada. Once this residue is methylated, Ada protein is converted into a transcriptional activator that stimulates transcription from its own promoter and from the *alkA* and *aidB* promoters [32,33]. Methylation of Ada protein is irreversible, that is, one Ada protein can repair only one lesion with each of its two methyltransferase activities, and it is not recycled.

The mechanism of transcription activation by Ada is complex because Ada interaction with RNA polymerase appears to be different at the *alkA* promoter from that at the *ada* and *aidB* promoters. The differences in Ada-dependent transcription among these three promoters have been examined in detail (for a review, see [48••]). A striking feature of the *ada* and *aidB* promoters is the presence of UP elements, DNA sequences that typically function as enhancers of transcription from factor-independent promoters [49–51]. The UP element is a binding site for the RNA polymerase (RNAP) α subunit, and the ability of α to bind to this sequence enhances transcription from the adjacent downstream promoter. The *ada* and *aidB* UP elements function as α-binding sites and enhance basal and ^{me}Ada-dependent transcription [48••,52–54].

At the *alkA* promoter, RNAP does not bind DNA in the absence of the Ada protein, because of the lack of a strong α-binding site [53]. Unlike its activity at the *ada* and *aidB*

promoters, Ada does not activate *alkA* transcription by RNA polymerase assembled with σ^S [55]. As a result, *alkA* expression is low in stationary phase even when the adaptive response is induced. These observations suggest that *alkA* may be less readily activated than *ada*. The differences between the *alkA* promoter and the *ada* and *aidB* promoters also extend to the Ada–RNAP interactions required for activation of transcription. Early studies of *ada* mutations have shown that determinants in Ada and RNA polymerase required for *alkA* induction are different from those involved in the induction of *ada*: only the amino-terminal domain of Ada is needed for *alkA* induction, whereas the carboxy-terminal domain is required for *ada* activation [56–58]. At the *ada* and *aidB* promoters, Ada contacts a set of negatively-charged amino acids in the σ^{70} subunit of RNAP. At the AlkA promoter, ^{me}Ada protein contacts a positively-charged patch in σ, and also interacts with the α subunit of RNAP [48••,59]. The positively-charged patch in σ^{70} required for interaction with Ada at *alkA* is not conserved in σ^S, providing the explanation for the lack of activation of RNAP–σ^S at the *alkA* promoter [54].

Repair of oxidative damage

Most genes encoding DNA repair enzymes that act on oxidative damage appear to be expressed constitutively in actively growing cells. This is presumably because oxidative DNA damage is continuously produced by reactive oxygen species (ROS), which are normal byproducts of aerobic metabolism [6,60]. However, in order to deal with elevated levels of peroxide in their environment, cells have evolved mechanisms to protect DNA from ROS. Most inducible genes that respond to oxidative damage prevent, rather than repair, DNA damage. A notable exception is endonuclease IV, an AP endonuclease that is also capable of repairing 3′ phosphate residues to 3′ OH groups that can prime DNA synthesis [61,62]. Consistent with a protective rather than repair function of the oxidative response genes, the signal triggering their induction is the presence of ROS, rather than the resultant DNA damage. Two key protective responses have been described in *E. coli* — one controlled by *oxyR*, the other by the *soxRS* genes [34,35,63]. These regulatory proteins are both transcription activators. OxyR is a member of the LysR family of regulatory elements, and SoxS is related to the AraC/XylS family of regulatory elements [64•].

Regulation of the SoxRS response to oxidative damage

The *soxRS* regulatory system acts in two steps with SoxR, serving both as a sensor and as an activator protein. When activated, SoxR induces transcription of *soxS*, a positive regulator that stimulates transcription of superoxide-responsive genes [65,66]. Both active and inactive SoxR protein binds between the –10 and –35 region of the *soxS* promoter. However, only binding by activated SoxR protein results in strong bending of the *soxS* promoter region; DNA bending repositions the –35 and –10 boxes of the *soxS* promoter, which are located at a suboptimal spacing of 19 nucleotides, allowing better recognition of the promoter by RNA polymerase [65,67–70]. Although this system responds to oxidative stress when cells are exposed to superoxide radical generating agents, it is not induced by H_20_2 [63,69,71]. Upon exposure of cells to agents that stimulate the formation of superoxide radicals, SoxR protein forms 2Fe–2S centers, which convert SoxR protein to a form active in transcription. Activated SoxR protein remains bound to its site on the *soxS* promoter, where it stimulates its own transcription and that of the *soxS* gene. SoxS protein then activates transcription of a set of genes that include superoxide dismutase, endonuclease IV, glucose-6-phosphate dehydrogenase, and a variety of efflux pumps involved in multi-drug resistance mechanisms [72–74]. Upon relief of oxidative stress, SoxR is rapidly converted to its transcriptionally inactive form, turning off the response [67,73,75–77].

The OxyRS response to oxidative damage

OxyR also acts as a sensor of the oxidative state of the cell, but unlike SoxR, it responds to H_20_2. H_20_2 activates the transcriptional activity of OxyR by oxidizing two of its cysteine residues [78,79••,80]. When activated, OxyR activates transcription of the oxidative stress genes under its control. This results in induction of genes that include *oxyS*, *katG* (which encodes the H_20_2 detoxification enzyme catalase-hydroperoxidase I), *ahpC* (which encodes alkylhydroperoxidase), *grxA* (which encodes glutaredoxin), *gorA* (which encdoes glutathione reductase), and *dps* (which encodes a protein that protects DNA from peroxide damage) [81,82]. The *oxyR* gene regulates some genes directly and others indirectly via its induction of the *oxyS* gene [83,84]. The mechanisms by which *oxyR* regulates *oxyS* and its own expression differ from the mechanisms of regulation of other genes under direct control of *oxyR*. The *oxyR* and *oxyS* promoters are transcribed in divergent directions and share a single binding site that accommodates two dimers of OxyR, either in the reduced or in the oxidized form. Binding of the reduced form of OxyR represses transcription of *oxyR* and *oxyS*. Upon oxidation, OxyR shifts its binding positions and causes induction of *oxyS* and continued repression of its own transcription [80]. *oxyS* encodes an untranslated mRNA that appears to regulate the expression of a number of additional genes, possibly by an antisense mechanism [85•]. At OxyR-regulated promoters other than *oxyR* and *oxyS*, only oxidized OxyR binds. When bound by OxyR, cooperative binding with RNAP occurs, suggesting that OxyR recruits RNAP to the promoter [80,86,87]. Based on studies using mutant forms of RNAP, the recruitment function of OxyR appears to be similar to that of many other regulatory proteins, such as Crp and AraC, that bind cooperatively with RNAP via contacts between the regulatory protein and the carboxy-terminal domain of the RNA polymerase α subunit [88].

Conclusions

The mechanisms of regulation of the DNA repair and protection responses described above use standard positive

and negative regulatory mechanisms to control gene expression. However, the regulatory molecules are complex. They are involved in unique signal transduction mechanisms that monitor the cell for damage to DNA or sense an environment that is potentially damaging. In the SOS and adaptive responses to methylation damage, in which gene expression and function have been most extensively studied, the expression of individual genes exhibits fine tuning. In the SOS response, such fine tuning is accomplished by promoters of different strengths and by different repressor-binding affinities [8]. However, additional post-transcriptional mechanisms also contribute by controlling the activity of key proteins that coordinate DNA repair with cell cycle regulation and mutagenesis [89•]. In the adaptive response to alkylation damage, fine tuning of gene expression is accomplished at the transcriptional level by different promoter structures, the presence and absence of UP elements, the nature of the interactions between the activator and RNAP, and promoter recognition by different σ factors. Future work is required to examine the detailed activator–polymerase–promoter interactions at oxidative response genes and to determine how individual genes may differ in their expression levels and timing, and how their expression may be modulated under different environmental and physiological conditions.

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