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Reconstitution of an SOS Response Pathway: Derepression of Transcription in Response to DNA Breaks

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Summary

***E. coli* responds to DNA damage by derepressing the transcription of about 20 genes that make up the SOS pathway. Genetic analyses have shown that SOS induction in response to double-stranded DNA (dsDNA) breaks requires LexA repressor, and the RecA and RecBCD enzymes—proteins best known for their role as initiators of dsDNA break repair and homologous recombination. Here we demonstrate that purified RecA protein, RecBCD enzyme, single-stranded DNA-binding (SSB) protein, and LexA repressor respond to dsDNA breaks in vitro by derepressing transcription from an SOS promoter. Interestingly, derepression is more rapid if the DNA containing the dsDNA break has a χ recombination hot spot (5′-GCTGGTGG-3′), suggesting a novel regulatory role for one of the most overrepresented octamers in the *E. coli* genome.**

Introduction

One of the most lethal types of DNA damage is the double-stranded DNA (dsDNA) break. In *Escherichia coli*, these potentially fatal lesions arise frequently during DNA replication (Michel et al., 1997) and, like other types of DNA damage, induce transcription of about 20 DNA repair and recombination genes in what is known as the SOS response (Walker, 1985). The central regulatory element of the SOS pathway is the LexA repressor, which binds to the operators of SOS genes with a high affinity, thereby inhibiting transcription (Brent and Ptashne, 1981; Little et al., 1981). Other requirements for induction of the SOS response depend on the type of DNA damage, but genetic analyses have shown that both the RecA and RecBCD enzymes—the two initiators of dsDNA break repair and homologous recombination—are essential for SOS induction in response to dsDNA breaks (Chaudhury and Smith, 1985).

In *E. coli*, dsDNA breaks are processed by the RecBCD enzyme. This multifunctional enzyme unwinds and simultaneously degrades DNA from a dsDNA end (Kowalczykowski et al., 1994; Taylor and Smith, 1985). Degradation is asymmetric, with the 3′-terminal strand at the entry end being degraded much more extensively than the 5′-terminal strand (see Figure 4) (Dixon and Kowalczykowski, 1993). Upon reaching a recombination hot spot, Chi (χ; 5′-GCTGGTGG-3′), in the proper orientation, the polarity of nuclease degradation is switched, and the 5′-terminal strand is degraded while the other DNA strand is preserved (Anderson and Kowalczykowski, 1997a). RecBCD enzyme then facilitates the loading of the homologous pairing and strand exchange protein, RecA, onto the ssDNA downstream of χ (Anderson and Kowalczykowski, 1997b). Since RecA protein competes poorly with SSB protein (Kowalczykowski et al., 1987), this coordinated loading of RecA protein by χ-activated RecBCD enzyme facilitates the formation of an active RecA nucleoprotein filament on the χ-protected ssDNA, despite the presence of SSB protein. This RecA nucleoprotein filament then promotes the pairing and exchange of DNA strands with a homologous DNA molecule.

In addition to promoting the pairing and exchange of DNA strands, in vitro studies established that single-stranded DNA (ssDNA) and a nucleotide cofactor activate the multifunctional RecA protein to stimulate the self-cleavage of LexA repressor (Little et al., 1980). The fact that noncleavable *lexA* mutants are constitutively repressed (Slilaty and Little, 1987), together with the genetic dependence on *recA* function for derepression of the SOS pathway, shows that cleavage of the LexA repressor by RecA protein is an essential step for induction of the SOS response.

Further definition of the mechanism of signal transduction mediated by DNA damage requires examination of both transcription and recombinational repair processes in vitro. Here we show that RecA protein, RecBCD enzyme, SSB protein, and LexA protein respond to the presence of dsDNA breaks by derepressing transcription from an SOS promoter. Interestingly, derepression is accelerated if the damaged DNA contains a χ site, suggesting a novel regulatory role for the recombination hot spot.

Results

Derepression of Transcription in Response to dsDNA Breaks

In order to examine the simultaneous behavior of recombination and transcription machinery, we first designed an in vitro system to assay for transcription from an SOS promoter. To this end, we fused the *recA* promoter region (which is regulated by the LexA repressor) to a G-less cassette (a region of DNA with no G's in the coding strand), and then cloned this construct into pUC19, now referred to as pUC19-SOS. The DNA sequence of this insert, and the downstream nucleotides of the plasmid, are shown in Figure 1. In vitro transcription using this covalently closed circular (ccc) template in the presence of rATP, UTP, and rCTP should lead to the production of a 56-nucleotide-long oligomer transcript, if transcription aborts at the first G of the coding sequence. Figure 2, lane b shows that transcription of this construct produces two specific transcripts: one that is the expected size and another due to transcription past the first G, which results from low amounts of contaminating rGTP in the purified rNTPs. Transcription from the pUC19 lacking the insert (Figure 2, lane a) produces

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Figure 1. Promoter Region of *recA* Gene Containing a G-less Cassette that Is under Control of the *lexA* Operator

The upstream 50 bp of the RecA promoter were fused to a 55 bp G-less cassette. The letters in italics represent the sequence of the surrounding pUC19 DNA. The termination points of transcription (i.e., the first G's after the start of transcription) are shown in bold.

truncated background transcripts, but nothing larger than a 30-mer, showing that the 56-nucleotide and larger transcripts are specific to the *recA* promoter-G-less cassette.

As expected for a gene controlled by the *recA* promoter, transcription of the G-less cassette is repressed by the LexA repressor (Figure 2, lane c). This repression is specific to the insert, since it does not affect production of the truncated transcripts that arise from other promoters on the pUC19 plasmid. The addition of both RecA protein and single-stranded DNA (ssDNA) lead to complete derepression (Figure 2, lane f). Neither RecA protein alone nor ssDNA alone is sufficient to derepress transcription (Figure 2, lanes d and e). These results indicate that the RecA protein, activated by ssDNA and NTP, is sufficient to induce derepression of the RecA promoter.

Next, we tested the ability of RecA protein, RecBCD enzyme, and SSB protein to derepress a LexA-controlled promoter in response to a dsDNA break. Figure 3A, lane c, shows that transcription is repressed in the presence of RecA protein, SSB protein, RecBCD enzyme, and LexA repressor; only the LexA repressor is required for repression (Figure 3A, lane b). The addition of linear dsDNA to the complete reaction leads to a rapid derepression of transcription (Figure 3A, lanes d–k). The addition of an equal amount of ccc dsDNA did not induce derepression (Figure 3A, lane a). Interestingly, derepression occurs more rapidly if the linear dsDNA contains χ sites (Figure 3A, compare lanes d–g with lanes h–k).

To show that derepression of the *recA* promoter is dependent on cleavage of LexA repressor, the reactions

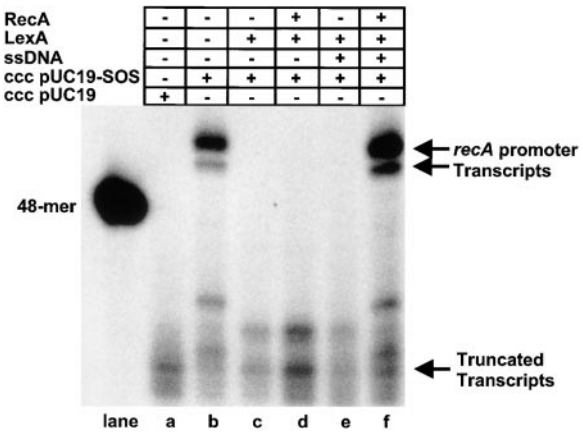

Figure 2. Derepression of the *recA* Promoter in Response to Activation of RecA Protein by ssDNA

Standard reaction conditions were used; specific components were omitted as indicated.

were repeated using LexA S119A protein, a noncleavable mutant LexA protein (Slilaty and Little, 1987). There was no measurable derepression in the presence of LexA S119A protein (Figure 3B, lane a). Furthermore, both RecA protein and RecBCD enzyme are required to derepress transcription in response to linear (i.e., broken) dsDNA (Figure 3B, lanes b and c). Finally, to further show that derepression of this system is rate-limited by dsDNA breaks, reactions were incubated with all components of the standard reaction, including RecA protein, RecBCD enzyme, SSB, and ccc pBR322 χ^+F,H, and then started by addition of ClaI restriction enzyme, which cuts pBR322 χ^+F,H but not pUC19-SOS. Incubation of the reaction with ClaI enzyme leads to derepression after 15 min (Figure 3B, lane e), while the same reaction without ClaI enzyme does not (Figure 3B, lane f), showing that these recombinational repair proteins mediate a response to dsDNA damage by derepressing LexA-controlled transcription.

Discussion

The repair of potentially lethal dsDNA breaks is crucial to the survival of all organisms. Optimal response to dsDNA breaks, like other environmental stresses, requires the ability to modulate levels of repair machinery in response to different types of DNA damage. Here, we describe the reconstitution of a complete in vitro system for signal transmission and transcriptional activation of an SOS gene in response to dsDNA breaks (see Figure 4). In this system, dsDNA breaks are detected by the RecBCD enzyme, which binds them specifically with a high affinity, and then unwinds and degrades the DNA 3′→5′; we imagine that other types of DNA damage, such as those produced by UV irradiation, either induce SOS by an independent pathway or are processed by other factors into dsDNA breaks. We show that the transcriptional response is regulated by the recombination hot spot, χ, which alters DNA processing in two significant ways. (1) After recognition of a χ site, the nuclease activity is reduced and the polarity of DNA degradation is switched. This leads to a net preservation of DNA (Anderson et al., 1997) and the production of an ssDNA overhang with χ at its 3′-terminus, which is the preferred DNA substrate for RecA protein (Tracy and Kowalczykowski, 1996) (Figure 4C). (2) In addition to this change in nuclease activity, RecBCD enzyme facilitates the loading of RecA protein on the ssDNA downstream of χ (Anderson and Kowalczykowski, 1997b). The activated RecA protein–ssDNA complex then stimulates the self-cleavage of LexA repressor and subsequent derepression of the SOS pathway.

The regulatory role of χ in the recombinational repair

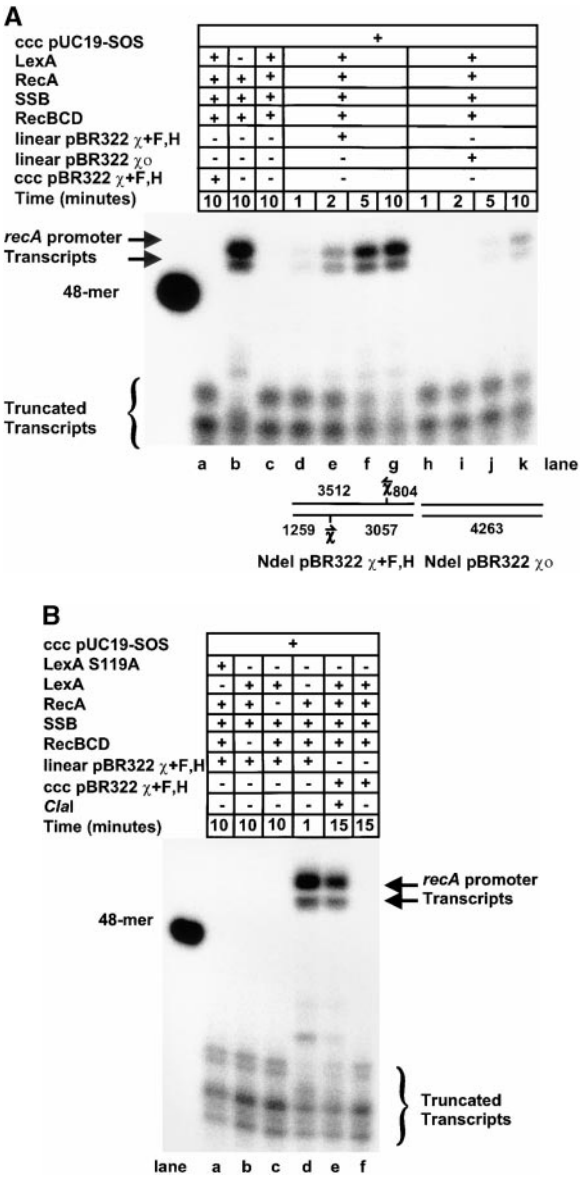

Figure 3. Derepression of Transcription in Response to dsDNA Breaks

(A) Derepression in response to χ. Reactions were initiated by addition of RecBCD enzyme or, when present, linear pBR322 DNA. (B) Derepression in response to a dsDNA break induced by ClaI restriction enzyme. Reactions were initiated with addition of linear pBR322 DNA or ClaI enzyme.

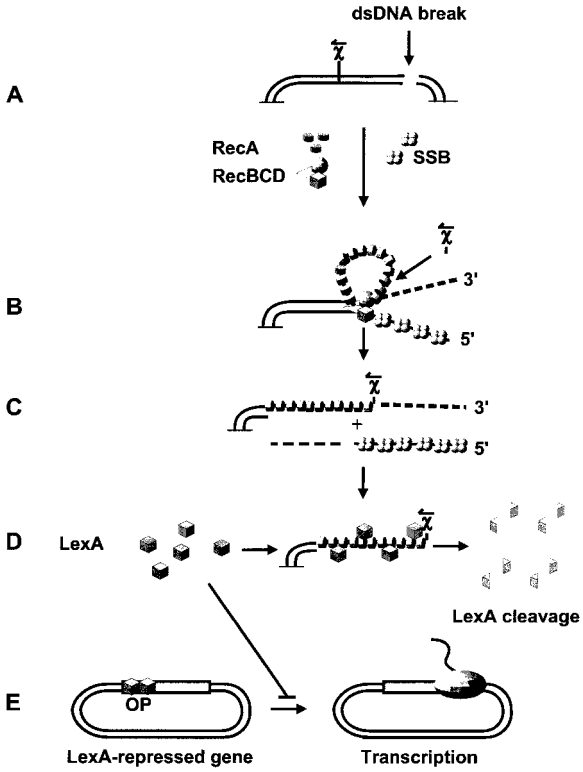

Figure 4. Model for the Transducing Signal Generated by a dsDNA Break to Derepression of the SOS Pathway by RecA Protein, RecBCD Enzyme, SSB Protein, LexA Repressor, and χ.

(A) DNA damage produces a dsDNA break.

(B) RecBCD enzyme processes the broken DNA, degrading 3′→5′ until a χ site is recognized, at which time RecBCD enzyme pauses; the 3′→5′ exonuclease activity is attenuated; and RecA protein is loaded onto the χ-containing DNA strand within an ssDNA loop.

(C) The nuclease polarity is then switched, with continued degradation occurring 5′→3′, leading to the production of a 3′ ssDNA overhang that is coated with RecA protein.

(D) This activated RecA nucleoprotein filament that is assembled on the χ-containing ssDNA stimulates the self-cleavage of LexA repressor.

(E) SOS genes are repressed by the binding of the LexA repressor to the operator (OP). The cleaved LexA protein no longer binds the promoter, thereby derepressing transcription.

of dsDNA damage has already been well documented: by inducing changes in RecBCD enzyme-mediated processing of DNA and loading of RecA protein, χ organizes the assembly of the recombinational repair machinery at the lesion (Eggleston and West, 1997). However, our data show that χ can also regulate repair at the transcriptional level. By facilitating the preservation of χ-containing DNA, the formation of an activated RecA nucleoprotein filament, and the subsequent cleavage of LexA repressor, χ ensures an efficient derepression of SOS repair genes in response to damaged DNA. Since χ is highly overrepresented in *E. coli* (Blattner et al., 1997) and is located within loci that are preferred targets for RecA protein binding (Tracy et al., 1997), we propose that this upregulation of the SOS pathway by χ insures that ample enzymatic repair machinery is induced to repair damage to "self" DNA, i.e., χ-containing DNA (Kuzminov, 1995; Myers and Stahl, 1994). Conversely, the less efficient SOS response to damaged "non-self" DNA, i.e., DNA without χ, such as the dsDNA of a virus or plasmid, routes processing of foreign DNA to a degradative, non-SOS-inducing pathway of DNA metabolism.

E. coli has evolved a relatively simple system for detection and response to DNA damage—the repair machinery plays a direct role in both the detection of and transcriptional response to DNA damage. This pathway is quite different from the elaborate kinase and transcriptional cascades that are responsible for response to DNA damage in eukaryotes (Weinert, 1998). However, it is interesting to note one parallel with the response

to dsDNA damage in *Saccharomyces cerevisiae*; Lee et al. (1998) recently suggested that the rate of ssDNA produced by 5′→3′ exonucleolytic degradation from dsDNA breaks controls escape from the G2/M checkpoint. Whether this or any other system in eukaryotes also possess a transcriptional response mechanism that can distinguish "self" from "non-self" DNA remains to be determined.

Experimental Procedures

Enzymes

RecBCD enzyme, RecA protein, and SSB protein were purified as described (Anderson and Kowalczykowski, 1997b). LexA repressor was purified as described (Harmon et al., 1996). LexA S119A protein was a kind gift from Dr. Hazel Holden (University of Wisconsin, Madison). RNA polymerase holoenzyme was a kind gift from Stacey Traviglia and Dr. Claude Meares (University of California, Davis). All restriction endonucleases and DNA-modifying enzymes were purchased from New England BioLabs.

DNA Substrates

The template plasmid pUC-SOS was created by inserting the oligo linker shown in Figure 1 into the HindIII, XbaI restriction sites of pUC19 (Sambrook et al., 1989). Plasmid pBR322 χ^+F,H is a modified form pBR322 χ^+F225 (Smith et al., 1981; Dixon and Kowalczykowski, 1993) that contains two χ sites in opposite orientations (see Figure 3A). M13 ssDNA was purified as described (Sambrook et al., 1989). All plasmid DNAs were purified by cesium chloride density gradient centrifugation (Sambrook et al., 1989).

Reaction Conditions

The standard reaction mixture consisted of 25 mM Tris acetate (pH 7.5), 8 mM magnesium acetate, 1 mM dithiothreitol, 1 mM phosphoenolpyruvate, 12 U/ml pyruvate kinase, 50 μg/ml BSA, 25 μM (nucleotides) yeast tRNA, 1 mM rATP, 200 μM UTP, 25 μM rCTP, 666 μCi/ml [α-^{32}P]rCTP, 25 μM (nucleotides) ccc pUC19-I template, 50 nM RNA polymerase holoenzyme and, when present, 100 nM LexA repressor or LexA S119A protein, 6 μM (nucleotides) pBR322 χ^o or pBR322 χ^+F,H (ccc or linear), 5 μM RecA protein, 2 μM SSB protein, 0.12 nM functional RecBCD protein, and 17 U/ml ClaI enzyme. Assays were performed at 37°C. The reactions in Figure 2 had 2 μM RecA protein and/or 5 μM (nucleotides) M13 ssDNA.

Reactions were allowed to incubate with all components except RNA polymerase for 5 min, and were then started by addition of pBR322 or *Cla*I restriction enzyme. When no pBR322 was present, reactions were started by addition of RecBCD enzyme. These were incubated at 37°C for the specified time, and then RNA polymerase holoenzyme was added and allowed to incubate for 2 min.

Analysis of Reaction Products

The reaction mixture (30 μl) was stopped by the addition of (60 μl) stop buffer (0.05 M EDTA, 99% Formamide) to halt the reaction. Samples were analyzed by electrophoresis in 8% denaturing polyacrylamide gels (8 M urea) for approximately 45 min at 46 V/cm in TBE (90 mM Tris-borate [pH 8.3], 0.5 mM EDTA). A 5′ end–labeled 48-mer deoxyribooligonucleotide was used as a size standard. The gels were analyzed on a Molecular Dynamics Storm 840 phosphoimager.

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References

Anderson, D.G., and Kowalczykowski, S.C. (1997a). The recombination hot spot χ is a regulatory element that switches the polarity of DNA degradation by the RecBCD enzyme. Genes Dev. *11*, 571–581.

Anderson, D.G., and Kowalczykowski, S.C. (1997b). The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a χ-regulated manner. Cell *90*, 77–86.

Anderson, D.G., Churchill, J.J., and Kowalczykowski, S.C. (1997). Chi-activated RecBCD enzyme possesses 5′→3′ nucleolytic activity, but RecBC enzyme does not: evidence suggesting that the alteration induced by Chi is not simply ejection of the RecD subunit. Genes Cells *2*, 117–128.

Blattner, F.R., Plunkett, G., 3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F. et al. (1997). The complete genome sequence of Escherichia coli K-12. Science *277*, 1453–1474.

Brent, R., and Ptashne, M. (1981). Mechanism of action of the lexA gene product. Proc. Natl. Acad. Sci. USA *78*, 4204–4208.

Chaudhury, A.M., and Smith, G.R. (1985). Role of Escherichia coli RecBC enzyme in SOS induction. Mol. Gen. Genet. *201*, 525–528.

Dixon, D.A., and Kowalczykowski, S.C. (1993). The recombination hotspot χ is a regulatory sequence that acts by attenuating the nuclease activity of the E. coli RecBCD enzyme. Cell *73*, 87–96.

Eggleston, A.K., and West, S.C. (1997). Recombination initiation: easy as A, B, C, D... chi? Curr. Biol. *7*, R745–R749.

Harmon, F.G., Rehrauer, W.M., and Kowalczykowski, S.C. (1996). Interaction of *Escherichia coli* RecA protein with LexA repressor. II. Inhibition of DNA strand exchange by the uncleavable LexA S119A repressor argues that recombination and SOS induction are competitive processes. J. Biol. Chem. *271*, 23874–23883.

Kowalczykowski, S.C., Clow, J.C., Somani, R., and Varghese, A. (1987). Effects of the Escherichia coli SSB protein on the binding of *Escherichia coli* RecA protein to single-stranded DNA: demonstration of competitive binding and the lack of a specific protein–protein interaction. J. Mol. Biol. *193*, 81–95.

Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D., and Rehrauer, W.M. (1994). Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. *58*, 401–465.

Kuzminov, A. (1995). Collapse and repair of replication forks in Escherichia coli. Mol. Microbiol. *16*, 373–384.

Lee, S.E., Moore, J.K., Holmes, A., Umezu, K., Kolodner, R.D., and Haber, J.E. (1998). Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell *94*, 399–409.

Little, J.W., Edmiston, S.H., Pacelli, L.Z., and Mount, D.W. (1980). Cleavage of the Escherichia coli lexA protein by the recA protease. Proc. Natl. Acad. Sci. USA *77*, 3225–3229.

Little, J.W., Mount, D.W., and Yanisch-Perron, C.R. (1981). Purified lexA protein is a repressor of the recA and lexA genes. Proc. Natl. Acad. Sci. USA *78*, 4199–4203.

Michel, B., Ehrlich, S.D., and Uzest, M. (1997). DNA double-strand breaks caused by replication arrest. EMBO J. *16*, 430–438.

Myers, R.S., and Stahl, F.W. (1994). Chi and the RecBCD enzyme of Escherichia coli. Annu. Rev. Genet. *28*, 49–70.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Slilaty, S.N., and Little, J.W. (1987). Lysine-156 and serine-119 are required for LexA repressor cleavage: a possible mechanism. Proc. Natl. Acad. Sci. USA *84*, 3987–3991.

Smith, G.R., Kunes, S.M., Schultz, D.W., Taylor, A., and Triman, K.L. (1981). Structure of Chi hotspots of generalized recombination. Cell *24*, 429–436.

Taylor, A.F., and Smith, G.R. (1985). Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*. J. Mol. Biol. *185*, 431–443.

Tracy, R.B., Chedin, F., and Kowalczykowski, S.C. (1997). The recombination hot spot chi is embedded within islands of preferred DNA pairing sequences in the E. coli genome. Cell *90*, 205–206.

Tracy, R.B., and Kowalczykowski, S.C. (1996). In vitro selection of preferred DNA pairing sequences by the *Escherichia coli* RecA protein. Genes Dev. *10*, 1890–1903.

Walker, G.C. (1985). Inducible DNA repair systems. Annu. Rev. Biochem. *54*, 425–457.

Weinert, T. (1998). DNA damage and checkpoint pathways: molecular anatomy and interactions with repair. Cell *94*, 555–558.