Volume 16 Number 20 1988 Nucleic Acids Research

Potential role of proteolysis in the control of UvrABC incision

Paul R.Caron+ and Lawrence Grossman

Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

Received June 16, 1988; Revised and Accepted September 23, 1988

ABSTRACT

UvrB is specifically proteolyzed in *Escherichia coli* cell extracts to UvrB*. UvrB* is capable of interacting with UvrA in an apparently similar manner to the UvrB, however UvrB* is defective in the DNA strand displacement activity normally displayed by UvrAB. Whereas the binding of UvrC to a UvrAB-DNA complex leads to DNA incision and persistence of a stable post-incision protein-DNA complex, the binding of UvrC to UvrAB* leads to dissociation of the protein complex and no DNA incision is seen. The factor which stimulates this proteolysis has been partially purified and its substrate specificity has been examined. The protease factor is induced by "stress" and is under control of the *htpR* gene. The potential role of this proteolysis in the regulation of levels of active repair enzymes in the cell is discussed.

INTRODUCTION

Proteolysis is known to have many important roles in the regulation of activity of many proteins in both prokaryotes and eukaryotes. These roles include zymogen activation, processing of signal sequences required for protein transport, and production of neurologically active peptides, in addition to the important role of proteolysis in protein degradation (1). In *E. coli*, proteolysis of several proteins involved in DNA replication and repair have been reported. The induction of the SOS system is dependent on the cleavage of the LexA repressor protein in a reaction contingent on activated RecA protein (2,3). The cleaved LexA protein has decreased binding affinity for DNA and RNA transcription is thus allowed. The SfiA protein (SulA) is one of the proteins induced following LexA cleavage. SfiA, which blocks cell division, is subsequently cleaved in an ATP dependent reaction by the Lon protease, resulting in resumption of cell division (4). Recently, proteolysis of the UmuD protein has been reported by activated RecA protein (5-7). UmuD appears to be effected by proteolysis at two stages: it is transcriptionally derepressed following LexA cleavage, and proteolysis of UmuD leads to activation of its activity. The *E. coli dnaX* gene encodes a single large open reading frame which produces two polypeptides, the τ and the γ subunits of DNA polymerase III holoenzyme (8,9). The γ subunit has been reported to arise from specific proteolytic processing of the τ subunit (10).

Proteolysis of UvrB to UvrB* has been shown in the accompanying paper (11) to alter the properties of the UvrB protein such that the UvrB* possesses DNA dependent ATPase activity which is sequestered in UvrB. Close examination of the properties of UvrB* versus those of UvrA and UvrAB led to the conclusion that the UvrB ATPase is required for DNA incision and is expressed only when bound to UvrA and DNA. The altered ability of UvrB* to hydrolyze ATP in

© IRL Press Limited, Oxford, England.

the absence of UvrA may be expected to alter its function in the UvrABC incision complex. Issues, such as whether the UvrB* can still interact with UvrA and/or UvrC to form a functional protein complex, can be addressed.

The UvrABC incision reaction has been divided into several partial reactions in order to examine the actions of the individual proteins in the ternary complex. Namely, the UvrA protein can bind specifically to damaged DNA (12,13). The UvrB protein can form a UvrAB complex which binds more tightly to damaged DNA (12,14). The UvrAB proteins are capable of topological unwinding of DNA (15) and of strand displacement of oligonucleotides from DNA in a helicase like reaction (16). DNA incision requires the addition of UvrC and can be measured either by formation of nicks in supercoiled DNA molecules or by analysis of the unique oligonucleotide product of this reaction (14,17). The functions of UvrB* will be examined using a number of these reactions in order to determine its functionality and to help determine the role of UvrB in the repair reaction and the possible significance of the proteolysis reaction. In addition the substrate specificity of the protease activity will be examined using the product of the *E. coli* *ada* gene, a bifunctional DNA alkyltransferase and transcriptional activator, as a potential substrate. Ada is rapidly proteolyzed in cellular extracts at two sites which have homology with the proposed cleavage site of UvrB (18,19).

METHODS

Strains

E. coli strain DH5α was obtained from BRL. Strains MH1 (*hfl*$^{+}$), X9368 (*hflA*::Tn5), HHC104 (*hflB*29 *zfi*25::Tn10), HHC108 (*hflA*::Tn5 *hflB*29 *zfi*25::Tn10) were obtained from Cheng and Echols (20). Strain AR68 (*htpR*$^{-}$) was obtained from M. Rosenberg.

Enzymes

The UvrA, UvrB, UvrC, and UvrB* were purified as previously described (11,21). The Ada protein was a generous gift of P. Robbins and T. Lindahl of the ICRF, UK.

Assays

All reactions were performed in a volume of 50 µl in 85 mM KCl, 40 mM morpholinopropanesulfonate (MOPS) at pH 7.6, 2 mM EDTA, 15 mM $MgCl_2$, 2 mM DTT. ATP was included at 2 mM where indicated. Nucleoside triphosphatase activity was determined by polyethylene imine (PEI) thin layer chromatography using [^{3}H]-GTP (ICN) as a substrate (11). UvrAB-DNA and UvrABC-DNA complexes were assayed by nitrocellulose filter binding after dilution with cold 2X SSC (12). UvrAB catalyzed strand displacement was measured by displacement of a 17-mer from M13 phage DNA (11). Damaged DNA incision of [^{3}H]-labeled supercoiled (RFI) plasmid DNA was measured by nitrocellulose filter binding after denaturation at pH 12.1 and renaturation (14). Nondamaged DNA incision was measured by scintillation counting of the oligonucleotide product after alkaline sucrose gradient fractionation and polyacrylamide gel electrophoresis (17).

Protease purification

E. coli cells were washed with 0.3 M NaCl, 100 mM Tris-HCl, pH 7.6, then lysed by addition of EDTA, DTT, and lysozyme followed by sonication as described (21). Cell debris was

Table 1. Effect of UvrB* on formation of a stable UvrAB-DNA complex

	fmol DNA bound		
	-UV+ATP	+UV+ATP	+UV-NTP
UvrA	5.8	9.8	2.9
UvrAB	29.8	40.4	3.1
UvrAB*	28.5	36.6	4.0

Table 1. Effect of UvrB* on the formation of a UvrAB-DNA complex. UvrA (100 nM) and UvrB (80 nM) or UvrB* (80 nM) were incubated with 66 fmol [^{3}H]-pPYC3 RFI DNA for 10 min. Irradiated DNA contained an average of 4 pyrimidine dimers per DNA molecule. The number of fmol of DNA bound to nitrocellulose after washing with 2X SSC is indicated. No significant binding of damaged or nondamaged DNA was seen with UvrB or UvrB* in the absence of UvrA.

removed by centrifugation at 20,000 x g for 40 min. Cellular membrane fractions were then isolated by centrifugation at 100,000 x g for 1 hr. These fractions were resuspended in Tris/NaCl buffer containing 1% Nonident P40 (Sigma), 0.2% Triton X-100 (BDH) for 1 hour. The solubilized protease was isolated after centrifugation at 150,000 x g for 2 hr. All steps were performed at 4°.

RESULTS

It has previously been observed (11,22) that UvrB is specifically proteolyzed to UvrB* in *E. coli* extracts. UvrB* is able to hydrolyze ATP in a UvrA independent, DNA dependent reaction. In order to determine whether UvrB* can functionally interact with UvrA in solution, the inhibition of the UvrA associated nucleoside triphosphatase activity was measured. Caron and Grossman (11) previously reported that UvrA protein is capable of hydrolyzing GTP and ATP with similar kinetics. The binding of UvrB has been shown to decrease both the ATPase and GTPase

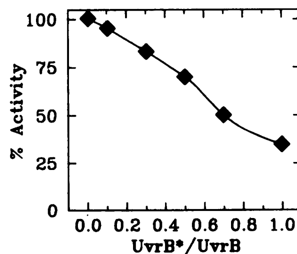

Figure 1. Inhibition of UvrAB strand displacement activity by UvrB*. The displacement of a 17-mer hybridized to M13 phage DNA was followed in a reaction containing UvrA (50 nM), UvrB (50 nM), ATP, and increasing concentrations of UvrB*. Activity remaining as a function of the ratio of UvrB to UvrB* is plotted.

activities of UvrA in the absence of DNA. According to this assay, UvrB* can bind to UvrA and can lead to a change in the properties of UvrA, similar those caused by UvrB binding, leading to UvrA nucleoside triphosphatase depression (data not shown).

Another characteristic of the UvrA-UvrB complex formation is its ability to bind DNA in a damage specific manner to nitrocellulose in the presence of 2X SSC (12). This binding, normally dependent on the presence of ATP, is similar with both UvrAB and UvrAB* (Table 1). Thus, UvrB* can bind to UvrA and a nucleoprotein complex with properties similar to a UvrAB-DNA complex can be formed.

The UvrAB proteins are able to displace short oligonucleotides which are hybridized to circular single strand DNA in the presence of ATP (16). The ability of a UvrAB* complex to catalyze this reaction was analyzed and found to be completely defective. Moreover, the UvrB* was found to inhibit the reaction normally obtained with the UvrAB proteins (Fig. 1) presumably by competition with UvrB in formation of the UvrAB complex.

The ability of UvrABC to incise, both damaged and undamaged DNA has been described previously (14,17,23). Briefly, the UvrABC endonuclease leads to the generation of an oligomer of 12-13 nucleotides in length with damaged DNA and approximately 9 nucleotides in length with nondamaged DNA. However, UvrAB*C is completely defective in the incision of either UV damaged or nondamaged DNA (Table 2). High levels of the UvrBC proteins lead to incision of nondamaged DNA in an ATP-dependent reaction yielding a 9 nucleotide oligomer (17). UvrB*C was defective in this incision as well.

Table 2. Effect of UvrB* on DNA incision.

	Relative DNA Incision	
proteins	UV damaged	Nondamaged
UvrC	0	8.0
UvrAC	0	8.0
UvrB*C	4.5	7.2
UvrBC	12.5	26.4
UvrAB*C	1.0	0.8
UvrABC	100.0	100.0

Table 2. Effect of UvrB* on DNA incision. UvrA (100 nM), UvrB (100 nM), UvrB* (100 nM), UvrC (6 nM) were incubated at 37° for 15 min with [^{3}H]-pPYC3 RFI DNA containing an average of 4 dimers/molecule and assayed for DNA incision by nitrocellulose filter binding after denaturation and renaturation. Alternatively, UvrA (100 nM), UvrB (100 nM), UvrB* (100 nM), UvrC (50 nM) were incubated at 37° for 15 min with linear [^{32}P]-pPYC3 DNA which was not irradiated. The formation of the oligonucleotide product of this reaction was quantitated by after isolation by polyacrylamide gel electrophoresis. Numbers in the table are relative to the incision seen with UvrABC. This represents 90% incision of UV damaged DNA and 0.15% incision of nondamaged DNA.

To further characterize the differences between the UvrB* and UvrB, the stability of the UvrAB and UvrABC complexes were analyzed when UvrB* substituted for UvrB (Fig. 2). The dissociation curves in 2X SSC at 0° for the UvrAB, UvrABC and UvrAB* DNA complexes indicate biphasic kinetics. The fast rate of approximately 1 min^{-1} may be due to dissociation of either UvrA complexes or abortive UvrAB complexes which did not properly form. The slow rate of dissociation is similar for UvrAB and UvrABC in this assay, 0.021 and 0.017 min^{-1} respectively. The slow rate of dissociation for UvrAB*, 0.092 min^{-1}, is approximately 4 times faster than that of UvrAB. The significance of this difference is not known. The most striking difference observed between complexes containing UvrB and UvrB* is the dissociation rate of UvrABC complexes versus UvrAB*C complex. The binding of UvrC to the UvrAB* complex leads to rapid dissociation of the complex from the DNA. The instability of DNA binding by UvrAB*C complex may explain the inability of UvrAB*C to incise DNA. The cellular significance of a protease activity in *E. coli* leading to the cleavage of UvrB to UvrB* was subject to further investigation, one reason being that should proteolysis of the UvrB occur, then the entire UvrABC complex is rendered nonfunctional since DNA binding is destabilized and DNA incision is eliminated.

The factor which stimulates the cleavage of UvrB in *E. coli* cell extracts was partially purified by detergent extraction of *E. coli* membranes (see Methods). This factor is stable in the presence of detergent, but is inactivated by most chromatographic techniques. The protease appeared to be very specific for UvrB. The UvrA and UvrC proteins were not cleaved by this protease nor were the mobilities of any of the bands from *E. coli* crude cell lysates affected by this protease fraction. The similarity of the proposed cleavage site of UvrB with the Ada protein cleavage sites suggested that Ada might by a substrate for this protease (19). It can be seen in Fig. 3 that the Ada protein (generously provided by P. Robbins and T. Lindahl) is specifically cleaved by this protease generating the expected products of 15, 19, 20, and 24 kD (18). Although the UvrB and the Ada protein both show sensitivity to a number of proteases in these regions (24), the cleavage by this protease was unique in that only the expected products were generated and that these products were stable even after incubations containing a 10-fold excess of protease.

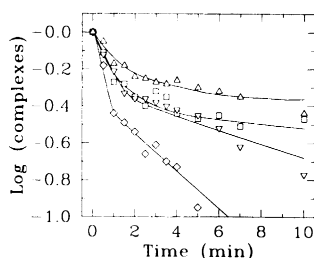

Figure 2. Dissociation kinetics of Uvr protein complexes. Uvr protein complexes were formed with UV irradiated RFI [^{3}H]-pPYC3 DNA (4 pyrimidine dimers per circle) at 37° for 15 min in the presence of ATP, then diluted with 100 volumes, 2X SSC, 0° for various lengths of time. The concentrations of the Uvr proteins before dilution were: UvrA - 10 nM; UvrB - 20 nM; UvrB* - 20 nM; UvrC - 20 nM. Data was plotted by calculating the fraction of total complexes remaining at a given time. △ - UvrA + UvrB; ▽ - UvrA + UvrB*; □ - UvrA + UvrB + UvrC; ◇ - UvrA + UvrB* + UvrC.

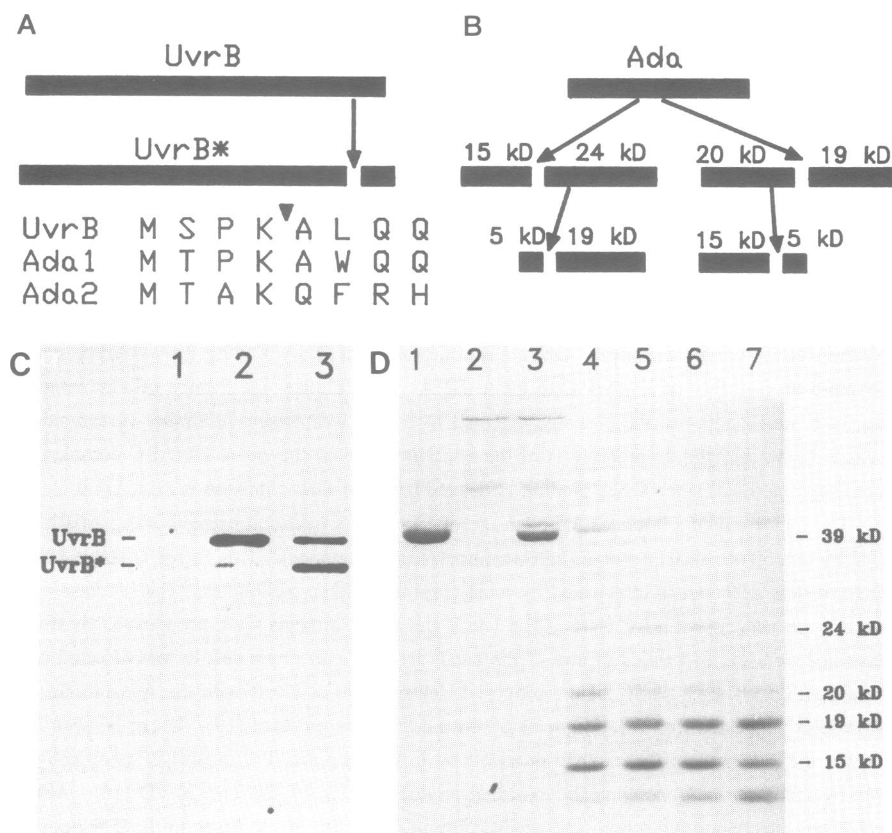

Figure 3. Cleavage of Ada and UvrB by detergent solubilized protease. A. Location of proposed cleavage site in UvrB and homology with the Ada protein. B. Expected products resulting from independent specific proteolysis of Ada at two sites (18). UvrB (5 µg) and Ada (5 µg) were proteolyzed using the solubilized protease preparation and the products analyzed by SDS-polyacrylamide gel electrophoresis. C. Proteolysis of UvrB, 7.5% polyacrylamide gel: lane 1, protease preparation; lane 2, UvrB + protease, 0°, 4 hrs; lane 3, UvrB + protease, 37°, 4 hrs. D. Proteolysis of Ada, 12% polyacrylamide gel: lane 1, Ada; lane 2, protease preparation; lane 3-7, Ada + protease incubated at 37° for 0, 6, 20, 30, and 60 min respectively.

As stated previously, the UvrB is specifically cleaved to UvrB* and the kinetics of this type of reaction show precursor-product kinetics (Fig. 4) consistent with earlier observations (22). The kinetics of cleavage of the UvrB protein examined at a constant concentration of protease factor exhibited an apparent K_m of 20 nM for UvrB. Competition experiments with the Ada protein indicate a similar affinity for the protease.

The protease was found to be induced 5-7 fold by heat shock at 42°. The induction of this protease could also be observed at 50° and by nalidixic acid (Fig. 5). These effects are consistent with the induction of the protease by the "heat shock" response. The induction by nalidixic acid is delayed as observed formerly for induction of heat shock genes by this SOS inducing treatment

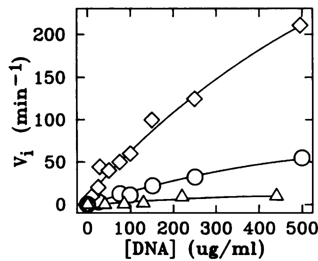

Figure 4. Kinetics of UvrB cleavage. The cleavage of UvrB (◆) and the production of UvrB* (◇) were followed by densitometry of Coomassie stained polyacrylamide gels.

(25). Furthermore, no induction of this protease activity was seen in strain, AR68, a htpR$^-$ strain. Normal protease induction was seen in *hflA*$^-$, *hflB*$^-$, or *hflA*$^-$*hflB*$^-$ strains which are deficient in the proteolysis of λcII and a number of *E. coli* proteins (20).

DISCUSSION

UvrB* is able to bind to UvrA and leads to changes in the UvrA structure which result in a decrease in UvrA nucleoside triphosphatase activity. This is identical to the effect seen with UvrB. The UvrAB* complex is capable of binding to DNA in an ATP and damage specific manner, similar to that observed for formation of UvrAB, although the UvrAB* complex dissociates at a rate which was slightly faster than that of UvrAB. The UvrAB* complex is defective in its ability to displace oligonucleotides compared to the UvrAB proteins. Observations in the preceding paper (11) indicate that the UvrB associated ATPase activity is essential for oligonucleotide displacement and one explanation for the defect seen with UvrB* is that its ATPase activity is now uncoupled from performing work. Subsequent steps in the UvrABC excision repair pathway are also inhibited. This is most likely due to the instability of the UvrAB*C complex on DNA.

The significance of proteolysis in *E. coli* is at this time still unknown. The UvrB specific

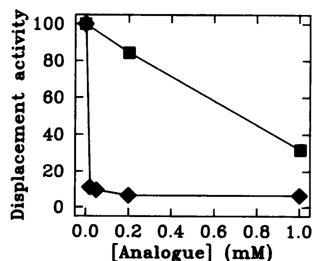

Figure 5. Induction of protease activity. Relative specific activity of the protease were determined in membrane fractions of strain MH1 after induction at time 0 with 50 μg/ml nalidixic acid at 25° (■) or after a temperature shift at time 0 to 50° (◆). Similar induction profiles were seen with all strains tested except for AR68 (*htpR*$^-$) which possessed a slightly depressed basal level of protease and no induction of protease activity at 37°, 42°, or 50°.

protease activity which has been partially purified in this paper was found to specifically cleave two proteins which are involved in DNA repair: UvrB and Ada. Cleavage of UvrB leads to functional inactivation of both the UvrA and UvrC proteins through protein complex formation. It has recently been described that the UvrABC proteins are able to incise DNA which is nondamaged (17). Cleavage of nondamaged DNA could lead to serious consequences for the cell, as it increases the risk of an abnormal completion of the repair reaction, such as failure to religate or increased mutation rate due to polymerase infidelity. Proteolysis of UvrB may be important in the prevention of an accumulation of excess levels of the UvrABC endonuclease. Another possible role for proteolysis is to allow turnover of UvrABC complexes which either form improperly or bind to sites which cannot be cleaved by UvrABC.

Another example of the need to prevent excess levels of repair proteins is the *ada* system. The Ada protein is a methyl-transferase with two active sites: one can remove methyl groups from O^6-methyl guanine and the other from methyl phosphates (24,26). Work in several laboratories has shown that the Ada protein does not turn over. Hence, mechanisms for indirect turnover through protein synthesis and degradation must be proposed. Ada which is methylated in the methyl phosphate acceptor site acts a positive regulator of its own synthesis as well as of a number of genes responsive to alkylating agents (27-29). The removal of nonmutagenic and nonlethal methyl phosphates is thus believed to be a way of monitoring the level of alkylation damage in the cell. Proteolysis of Ada is proposed to be involved in removing the methylated protein and turning off the induction of the alkylation genes (27). The cleaved protein retains the ability to accept methyl groups from DNA at both active sites, but is no longer able to induce Ada mRNA synthesis. The adaptive response to alkylation damage and the SOS response to bulky adduct damage may represent overlapping pathways as alkylating agents are able to induce the SOS response in mutants which are deficient in alkylation repair (30,31), and these mutants are dependent on the Uvr genes for survival (32).

The regulation of the protease factor is consistent with that of other heat-shock genes under control of the *htpR* gene (33). It is unclear whether this protein is a direct product of a heat shock gene or is induced indirectly by the heat shock regulon. The function of the heat shock proteins is unclear as these proteins can be induced by a number of treatments, and can generally be referred to as "stress" induced proteins (34). These proteins have previously been reported to be induced as a delayed response to DNA damaging agents which normally induce the SOS response (24). A role of a heat shock protein in turning off a SOS induced gene has already been described; the lon protease leads to the cleavage of a number of proteins including SfiA, which is a protein induced by SOS that inhibits cell division (35). The protease which cleaves Ada and UvrB is probably not the lon protease as the protease activity was found in lon^- cells (18) and was found not to be ATP dependent and has a different pH optimum. The proposed role of this protease, to turn off various DNA repair responses, is however similar to that of lon.

The finding that the protease responsible for UvrB cleavage is membrane bound is in accord with other findings which suggest that DNA replication and repair in bacteria may be membrane bound (36-40). A large fraction of the RecA protein has been found to be bound to membrane fractions of SOS induced *E. coli* (41). This is accompanied by a change in the levels

of several outer membrane proteins, the significance of which is unknown. Hydrolysis of ATP by the DNA replication origin binding protein of *E. coli*, dnaA, has been reported to be specifically stimulated by cardiolipin, a major component of the cellular membrane. The SfiA protein, described above, is attached to an integral membrane protein, SfiB (35). Interestingly, the cleavage of UvrB to UvrB* results in a change in the hydrophobicity of UvrB, such that although UvrB binds tightly to a phenyl agarose column, the UvrB* does not.

Recently, a gene which is involved in DNA repair in yeast, RAD6, has been found to encode a ubiquitin conjugating enzyme; its specificity and exact role in DNA repair is not clear (42). Other studies in eukaryotes indicate that ubiquitin conjugation is generally regarded as a method of targeting proteins for proteolytic digestion, possibly a direct result of ubiquitin (43).

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institutes of Health (5ROI-GM22846) and Department of Energy (DE-FG02-86ER60396). Work done by LG in the laboratory of T. Lindahl, ICRF, UK, was supported by the Burroughs Wellcome Travel fund.

+Present address: Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA

REFERENCES

1. Bond, J. S. and Benyon, R. J. (1987) *Molec. Aspects Med.* **9**, 173-287.
2. Little, J. W. (1983) *J. Mol. Biol.* **167**, 791-808.
3. Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60-93.
4. Mizusawa, S. and Gottesman, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 358-362.
5. Shinagawa, H., Iwaski, H., Kato, T., and Nakata, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1806-1810.
6. Burckhardt, S. E., Woodgate, R., Scheuermann, R. H., and Echols, H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1811-1815.
7. Nohmi, T., Battista, J. R., Dodson, L. A., and Walker, G. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1816-1820.
8. Yin, K.-C., Blinkowa, A., and Walker, J. R. (1986) *Nucleic Acids Res.* **14**, 6541-6549.
9. Flower, A. M. and McHenry, C. S. (1986) *Nucleic Acids Res.* **14**, 8091-8101.
10. Lee, S.-K., Kanda, P., Kennedy, R. C., and Walker, J. R. (1987) *Nucleic Acids Res.* **15**, 7663-7675.
11. Caron, P. and Grossman, L. (1988) preceding article.
12. Yeung, A. T., Mattes, W. B., and Grossman, L. (1986) *Nucleic Acids Res.* **14**, 2567-2582.
13. Seeberg, E. and Steinum, A.-L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 988-992.
14. Yeung, A. T., Mattes, W. B., Oh, E. Y., and Grossman, L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6157-6161.
15. Oh, E. Y. and Grossman, L. (1986) *Nucleic Acids Res.* **14**, 8557-8571.
16. Oh, E. Y. and Grossman, L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3638-3642.
17. Caron, P. and Grossman, L. (1988) *Nucleic Acids Res.* **16**, 7855-7865.
18. Teo, I. A. (1987) *Mutation Research* **183**, 123-127.
19. Arikan, E., Kulkarni, M. S., Thomas, D. C., and Sancar, A. (1986) *Nucleic Acids Res.* **14**, 2637-2650.
20. Cheng, H. H. and Echols, H. (1987) *J. Mol. Biol.* **196**, 737-740.
21. Yeung, A. T., Mattes, W. B., Oh, E. Y., Yoakum, G. H., and Grossman, L. (1986) *Nucleic Acids Res.* **14**, 8535-8556.
22. Thomas, D. C., Levy, M., and Sancar, A. (1985) *J. Biol. Chem.* **260**, 9875-9883.
23. Sancar, A. and Rupp, W. D. (1983) *Cell* **33**, 249-260.
24. Sedgwick, B., Robins, P., Totty, N., and Lindahl, T. (1988) *J. Biol. Chem.* **263**, 4430-4433.

25. Krueger, J. H. and Walker, G. C. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1499-1503.
26. Demple, B., Sedgwick, B., Robins, P., Totty, N., Waterfield, M. D., and Lindahl, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2688-2692.
27. Teo, I., Sedgwick, B., Kilpatrick, M. W., McCarthy, T. V., and Lindahl, T. (1986) *Cell* **45**, 315-324.
28. Nakabeppu, Y. and Sekiguchi, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6297-6301.
29. Lemotte, P. K. and Walker, G. C. (1985) *J. Bacteriol.* **161**, 888-895.
30. Costa de Oliveira, R., Laval, J., and Boiteux, S. (1986) *Mutation Research* **183**, 11-20.
31. Otsuka, M., Nakabeppu, Y., and Sekiguchi, M. (1985) *Mutat. Res.* **146**, 149-154.
32. Van Houten, B. and Sancar, A. (1987) *J. Bacteriol.* **169**, 540-545.
33. Grossman, A. D., Straus, D. B., Walter, W. A., and Gross, C. A. (1987) *Gen. Devel.* **1**, 179-184.
34. VanBogelen, R. A., Kelley, P. M., and Neidhardt, F. C. (1987) *J. Bacteriol.* **169**, 26-32.
35. Huisman, O., DAri, R., and Gottesman, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4490-4494.
36. Hendrickson, W. G., Kusano, T., Yamaki, H., Balakrishnan, R., King, M., Murchie, J., and Schaechter, M. (1982) *Cell* **30**, 915-923.
37. Hoshino, T., McKenzie, T., Schmidt, S., Tanaka, T., and Sueoka, N. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 653-657.
38. Mosig, G. and Macdonald, P. (1986) *J. Mol. Biol.* **189**, 243-248.
39. Todo, T. and Yonei, S. (1983) *Mutation Research* **112**, 97-107.
40. Todo, T., Yonei, S., and Kato, M. (1983) *Biochem. Biophys. Res. Comm.* **110**, 609-615.
41. Garvey, N., St. John, C., and Witkin, E. M. (1985) *J. Bacteriol.* **163**, 870-876.
42. Jentsch, S., McGrath, J. P., and Varshavsky, A. (1987) *Nature* **329**, 131-134.
43. Fried, V. A., Smith, H. T., Hildebrandt, E., and Weiner, K. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3685-3689.