COO/2814-3

MOLECULAR BASIS FOR THE MUTAGENIC AND LETHAL EFFECTS OF ULTRAVIOLET IRRADIATION

Progress Report

for Period December 1, 1977-November 30, 1978

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July 1978

Prepared For

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
UNDER CONTRACT NO. EY-76-S-02-2814

DISCLAIMER

SUMMARY OF PROGRESS

A.4. Comprehensive Progress Report

a. Period (1968 to Present)

b. Summary

Our earlier work on the chemical basis of mutagenesis led to certain chemical generalities necessary to explain how certain mutagens such as UV light and hydroxylamine functioned in information transfer systems (replicative, transcriptive and translational). When such modifications were applied to biologically active DNA in a controlled manner biological expression was non-stoichiometric because much of the damage was removed from the DNA by repair systems. Our efforts were then directed to these systems which led to:

1) The isolation, purification and characterization of endonucleases responsible for the first and controlling step in DNA repair - referred to as incision in both M. luteus and E. coli; ~~The biological role of these enzymes was inferred in appropriate mutants~~.

2) The isolation, purification and characterization of exonucleases responsible for the removal or excision of damaged nucleotides in M. luteus and human placental trophoblasts;

3) The repair of UV damaged biologically active transforming and transfecting DNAs by purified endonucleases, exonucleases, DNA polymerase I and polynucleotide ligase from M. luteus and E. coli.

4) The characterization of the dual gene control for incision phenomenon in M. luteus and E. coli; and

5) Isolation, purification and characterization of repair enzymes from human placenta, ~~(currently in progress)~~.

a) Ap endonuclease (apurinic/apyrimidinic ~~endonuclease~~) - a 32,000 m. wt. dimer endonuclease purified to homogeneity which acts only on sites in DNA lacking a pyrimidine/purine base.

b) Correxonuclease - a 30,000 m. wt. exonuclease from nuclear chromatin which possess excision and editing functions in repair.

c) β-DNA polymerase - a repair polymerase purified to homogeneity from chromatin which reinserts nucleotides during repair.

d) & e) A single stranded endonuclease from nuclei which acts on UV damaged DNA specifically in the presence of a binding protein which binds specifically to UV irradiated native DNA.

~~f) Polynucleotide ligase is currently being purified.~~

460263

Detailed Progress Report

I. Mutagenesis - (Refs. 1-12)

After extensive studies we were able to come to the general conclusion that the effective 5,6-reduction of cytosine residues in polynucleotides, DNA and as ribo-and deoxyribonucleotide triphosphates led to transitions in recognition at the level of replication, transcription and translation in vitro. The reduction of cytosine residues to 5,6-dihydrocytosine or to the cytosine water photoaddition product results in a change in the K_T (tautomeric constant) from 10^5 to that of unity. Reaction of cytosine nucleotides or polynucleotides with the mutagen hydroxylanine (NH_2OH) results in an identical change in the cytosine K_T from the dominant enamine to enimine form. These tautomeric shifts account for the in vitro transitional changes in recognition by polymerases and translating systems.

Similar structural changes to uracil and thymine residues leads to loss of hydrogen binding properties rather than changes in complementarity. K_T and pKa changes accompanying effective reduction of the 5,6 double bond support the biochemical refractoriness of these analogs.

Attempts were made to extend these studies to biological systems in which a correspondence between the numbers of cytosine residues quantitatively modified with ^{14}C-NH_2OCH_3 and specific gene expression was studied. It was found that there was little correspondence between transforming DNA modification and mutation because much of the damage that was introduced into DNA was removed by endogenous repair enzymes.

It was at this time that Setlow (Carrier and Setlow, PNAS 51, 226, 1967) and Howard-Flanders (Boyce and Howard-Flanders, PNAS 51, 293, 1964) simultaneously reported the excision of pyrimidine dimers from UV irradiated bacteria. Our interests then turned to the enzymology of DNA repair.

II. Early Steps in Repair - Procaryotes (13-36)

Introduction

The initiation of our enzyme studies was carried out with ultraviolet irradiated DNA substrates in which enzymes were isolated and characterized from M. luteus. Since the photochemistry of DNA had been sufficiently well characterized the temporal fate of photoproducts was amenable to study. M. luteus was chosen as a biologic source material after examining some 26 different bacterial species for ultraviolet resistance and their endogenous non levels of specific nucleases. The disadvantage with this organism was its lack of genetic characterization, specific bacteriophage and systems amenable for testing the biological activity of its DNA. The latter two problems were resolved after phage were isolated and characterized (24) and a transforming system established (13). Initial experiments with the genetically characterized E. coli were feasible after technical capabilities with M. luteus had been obtained (22,31). The disadvantage of E. coli as a source of repair enzymes is the limited

460261

number of gene copies. For this reason the genes uvrA, uvrB, and uvrC have been cloned and are currently being prepared, and analyzed for gene amplification purposes.

Incision Step in Nucleotide Excision (15-24,31-34)

The initial step in the enzymatic removal of pyrimidine dimers from UV irradiated DNA is controlled by two genes in E. coli (uvrA, 91' and uvrB 19'), at least two in M. luteus and one in T_4 infected E. coli (V gene). In all cases the enzyme(s) effect a phosphodiester bond break 5' to the dimer on the damaged strand generating a 3'-OH and 5'-phosphoryl group.

In those E. coli or M. luteus mutants lacking one or the other incision genes there is no incision event. Two gene products have been isolated from M. luteus identified as correndonucleases I and II which correspond chromatographically to the uvrA and uvrB gene products respectively. Why two genes are required for what appears to be a simple phosphodiester bond hydrolytic step is subject to current study. It is apparent, however, that the resolved enzymes generate different sites on UV incised DNA. The basis for the cooperative requirement for both enzymes is potentially resolvable according to current experimental protocols.

The isolation of similar enzymes from eukaryotes has not been substantially demonstrated.

Excision Step in Prokaryotes (14-19,26-27)

The site which is generated by incision is sensitive to the action of either DNA polymerase I, DNA polymerase III or the UV exonuclease isolated from M. luteus or its equivalent from E. coli - exonuclease VII, isolated by Chase and Richardson (JBC.249, 4553 [1974]).

Those polymerase unassociated exonucleases act on single stranded DNA by initiating hydrolysis with equal facility from either the 3' or 5' terminus, liberate 5'-phosphorylated tetra and pentradeoxynucleotides from either terminus and can participate in repair by virtue of their ability act at internal rather than terminal phosphodiester bonds. These enzymes, although refractory to DNA duplexes, can initiate hydrolysis in the 5' direction of UV irradiated incised DNA duplexes. It presumably can act at such sites by virtue of the single stranded nature of the terminus which bears the non-hydrogen bonding pyrimidine dimers. As a consequence an octa - to deca nucleotide is initially generated. This small fragment is subsequently digested to a limit pentanucleotide by this exonuclease.

Excision of DNA Polymerase I of M. luteus (23,26,28,37)

DNA polymerase I of M. luteus like that of E. coli is a multifunctional single polypeptide of 109,000 molecular weight. In addition to its polymerizing activity it has a 3'→5' exonuclease which prefers single stranded DNA and acts at terminal non-esterified

phosphodiester bonds. It functions in "error-correcting" or "editing" during polymerization by removing non-complementary nucleotides from the 3' terminus of DNA (Brutlag and Kornberg, JBC 247, 247, 1972). The 5'→3' exonuclease prefers to initiate hydrolysis at internal phosphodiester bonds of duplex DNAs at nicks generated by non-specific endonucleases or those involved in incision reactions at damaged nucleotide sites. A defined stoichiometry is observed between the number of dimers, incised sites and DNA polymerase I molecules acting on UV irradiated transforming DNA which allowed for an analysis of the fidelity of repair processes in vitro. Such studies also provided some insights into the role of polynucleotide ligase in limiting the strand displacement reaction of DNA polymerase I to nick translation.

By using highly purified preparations of the pyrimidine dimer specific endonuclease, DNA polymerase I and polynucleotide ligase, which are virtually sterile, it was possible to restore the biological activity to UV inactivated B. subtilis transforming DNA under conditions in which the stoichiometry of each step in repair could be monitored.

Preexcision Step in Repair - Role of uvrC

Although the incision produced by the pyrimidine dimer specific endonucleases provide an initiation site for excision and nucleotide incorporation by pol I, indirect evidence suggests that an intermediate step between incision and excision exists. This evidence is based on the properties of the excision defective uvrC mutant. Such mutants are unable to remove pyrimidine dimers from DNA in vivo although there are indications that single strand incision events are operative.

Seeberg and Rupp (Molecular Mechanisms For Repair of DNA - Hanawalt and Setlow, eds., pg. 439) prepared a double uvrC lig ts mutant. At temperatures restrictive for ligase, the early rate and extent of postirradiation molecular weight losses are similar to tthose of wild type cells followed by the expected accumulation of low molecular weight DNA. At permissive temperatures, however, the molecular weight of the DNA is rapidly and fully restored. These data, in conjunction with the observations that ligase is capable of resealing the phosphodiester bonds of incised DNA, places ligase in a controlling position during early repair steps.

More recent data supporting the controlling role of ligase in the early repair steps was obtained by Sharman and Moses (in press) in which NMN led to the accumulation of single strand breaks in toluenized uvrC mutants after irradiation. In the absence of this ligase inhibitor there was evidence of incision during postirradiation conditions.

The isolation of the uvrC gene product is currently underway in the laboratory with the cloning of this structural gene on col EI plasmids. Amplification of the uvrC gene, inserted into the lac region of lambda, will permit further examination of this problem in greater detail at the molecular level.

Nucleotide Excision in Eukaryotes:

Introduction - Choice of Tissue

Human placenta provides a biochemically amenable, safe and plentiful source of human cells which exist primarily as syncytial trophoblasts of the fetal placental barrier. The trophoblasts do not divide, but possess protein and hormone synthesizing functions. These activities are necessary for normal gestation and are dependent on an intact genome maintained by active DNA repair systems. We have shown that the Ap endonuclease (apurinic/apyrimidinic),,correxonuclease, β-DNA polymerase, UV specific binding protein and single stranded endonuclease, all potential repair enzymes are located in trophoblast nuclei. Initially our experiments demonstrated the presence of all ingredients necessary for carrying out the excision and repair replication steps for ultraviolet and alkylated DNA in a system in vitro.

Incision Step

Ap Endonuclease (Apurinic (Apyrimidine Endonuclease) (38)

This endonuclease incises double stranded DNA, into which apurinic sites have been introduced, either as a result of spontaneous depurination or as a result of treatment of the DNA with alkylating agents, nitrous acid or ionizing radiation. The Ap endonuclease does not hydrolyze native DNA, alkali-denatured DNA, single stranded DNA containing apurinic sites or RNA. The incision is introduced at the 3' side residues generating a 3' hydroxyl and 5' phosphoryl group.

Ap Endonuclease Purification Scheme

1. Placental homogenate - major activity found loosely bound to nuclei, gently removed into a supernatant fraction.

2. Supernatant fraction applied to a column containing octylsuccinic anhydride coupled by a 1,6-diaminohexane spacer arm to Sepharose 4B. This ligand removes all of the serum albumin, a major contaminating protein.

3. Preparative isoelectric focusing - pI 7.6

4. Sephadex G-75

5. Hydroxylapatite gradients - homogeneous at this step with an overall 40% yield from the crude supernatant.

Assay - Conversion of partially-depurinated-(^{3}H)-ØX174-RF-I-DNA to the RFII species.

Protein Properties

Molecular Weight	Native	32,000
	Denatured	16,000
Isoelectric Point		7.6
$T_{½}$ @ 49°		3.5 mins.

460257

Excision Step

Human Placental Correxonuclease (30,35)

This is an exonuclease which acts specifically on single stranded DNA when compared to uninterrupted DNA duplexes. The enzyme initiates hydrolysis at both 3'- and 5'- termini of single stranded DNA yielding 5'-phosphorylated tetra- and pentadeoxynucleotides from either terminus. Because the enzyme hydrolyzes internal rather than terminal phosphodiester bonds the rates and extent of hydrolysis catalyzed by the correxonuclease are unaffected by damaged nucleotides. It is to be expected, ( )(like similar bacterial enzymes), that the correxonuclease can initiate hydrolysis at single strand breaks in irradiated incised DNA. Although not detectably active against intact native DNA, the correxonuclease can initiate hydrolysis in a 5'- and 3'- direction at single strand breaks creating a 30-40 nucleotide cavity. The presence of a pyrimidine dimer or an apurinic site at such a break is effectively excised by virtue of the enzyme's ability to act at internal phosphodiester bonds. The presence of non-complementary nucleotides on the 3' side of the break is also sensitive to the action of this exonuclease. The number of different properties associated with this single protein is reminiscent of the multicatalytic properties of the bacterial DNA polymerase I species of enzymes. By virtue of its 3'- and 5'- exonucleolytic activities this enzyme in addition to its polymerizing properties is capable of both "editing or proof-reading" of mispaired nucleotides and excision of those that are damaged.

Enzyme Purification Scheme

1. Placental homogenate - supernatant fraction stored
2. Supernatant fraction concentrated by polyethylene glycol dialysis
3. DEAE Chromatography (removes hemoglobin and nucleic acids)
4. Heparin-Sepharose Chromatography (affinity chromatography)
5. Hydroxylapatite Chromatography (resolved from most nucleases)
6. Sephadex-G-75 (Exclusion for similar molecular weight species)
7. Multiphor Isoelectricfocusing (Ip. 6.2 species - pure protein)

Protein Properties

1. Molecular weight	35,000
2. Isoelectric point	6.2
3. $T_{\frac{1}{2}}40^{o}$ -	20'
4. Km (for terminus) $\sim$	1×10^{-9} m

Insertion Reaction

DNA Polymerase (β)

A low molecular weight DNA polymerase (β) is present in the nucleus of placental trophoblasts. The level of this enzyme is not related to replication and its general properties suggest repair function. It has been estimated that there are 7.4×10^{4} molecules per cell with a polymerization rate of 2.5 nucleotides/molecules/second. It is the major DNA polymerase found in the non-dividing placental syncytial trophoblasts.

The enzyme incorporates nucleotides into double stranded DNA "gapped" by treatment with pancreatic DNase I and exonuclease III, and also into synthetic polydeoxyribonucleotides initiated with the complementary oligodeoxyribonucleotides. Its activity is dependent on the divalent cations Mg^{++} (5-12mM) or Mn (0.1-0.5mM) and the complementary deoxyribonucleoside triphosphate, although with a single deoxyribonucleoside triphosphate a limited end addition is possible. With synthetic homopolymer primer templates Mn^{++} is the preferred cation. The pH optimum varies between 8.0-9.0 depending on the DNA template and the buffer, and its activity is stimulated two fold by 100-200mM KCl.

Enzyme Properties:

The Kms for the deoxyribonucleoside triphosphate depends on the template and activating cation. A Km of 160 M has been found for activated DNA with Mg^{++}. For the synthetic polynucleotide template $dpA_{\overline{600}} - dpT_{\overline{37}}$ with Mn^{++} as the cation, the values varied from 28-42μM. The fidelity of copying a synthetic template poly d(A-T) using dCTP as the competing nucleotide is 1/25,000 as compared with 1/184,000 by N-ethylmaleimide and does not require a free thiol group for activity. There are no associated exonuclease activities, and it does not catalyze pyrophosphate exchange or pyrophosphorolysis of DNA dependent deoxyribonucleoside triphosphate degradation. There is no associated RNase H activity.

Protein Properties:

The enzyme is a basic protein with an isoelectric point of 9.2-9.4 and contains Zn^{++} associated with the protein molecule. It consists of a single polypeptide chain of molecular weight 45,000 daltons (3.5S), is stable in 5M urea at pH 4.5, and migrates as a cation on electrophoresis under these conditions.

Purification:

1) Subcellular fractionation: Nuclei are isolated from human placenta taken within 30 minutes of delivery. The enzyme is extracted from the washed nuclei with 0.2M potassium phosphate pH 7.5, 10mM mercaptoethanol 0.3M sucrose.

2) The nucleic acids are removed from the extract by passage through DEAE cellulose.

3) The nonadsorbed fraction is dialyzed and separated on phosphocellulose (P-11) by differential salt elution into an exonuclease (300mM) and a DNA polymerase (650mM) fraction.

4) The (β) DNA polymerase is separated from the (α) replicative enzyme in the DNA polymerase fraction, by chromatography on DEAE cellulose. The (β) enzyme passes through and the (α) is adsorbed.

5) The enzyme is dialysed and concentrated on a second phosphocellulose column, a step which also removes residual exonucleases.

6) Remaining protein containmants of high molecular weight are removed by gel filtration through Sephadex G100, and the enzyme of molecular weight 45,000 concentrated with ammonium sulphate.

7) The concentrated enzyme is dialyzed and applied to an affinity DNA cellulose column and eluted by salt.

8) The eluted enzyme dialyzed and finally purified by isoelectric focusing at a pI of pH 9.2-9.4. The enzyme is free of endonuclease activities and is stored in 50% glycerol at -20°.

Sealing of the Repaired Strand

Polynucleotide ligase is currently being purified according to established procedures (Lindahl, T., Methods in Enzymology 21, 33 (1971) and Spadori, et al Eur. J. Biochem. 22, 75 (1971).

Use of These Enzymes

By purifying the enzymes involved in a single pathway of DNA repair it is planned to study the mechanism, stoichiometry and fidelity of repair with biologically active DNA modified by specific depurination and depyrimidination reactions. These studies are supported by other granting agencies (American Cancer Society and the National Science Foundation).

SUMMARY OF PERTINENT DATA IN PREPRINT

Through the use of the pyrimidine dimer specific endonucleases of M. luteus it has been possible to identify the distribution of pyrimidine dimers in irradiated fragments of DNA with a defined sequence. Since then enzymes catalyze incisions at all pyrimidine dimers it has been used diagnostically for the nature of damage distribution according to sequence..

The results of our findings are that:

1) Damage is non-random.

2) The formation of all species of pyrimidine dimers is thymine dependent.

3) An alternating Py Pu Py Py TT Pu Py Pu Py sequence leads to higher dimer yields than predicted from (2) and may represent "hot spots" for damage.

REFERENCES

1. Grossman, L. The Effects of Ultraviolet Irradiated Polyuridylic Acid in Cell-free Protein Synthesis in E. coli. Proc. Natl. Acad. Sci. US 48, 1609 (1962).

2. Grossman, L. The Effects of Ultraviolet-irradiated Polyuridylic Acid in Cell-free Protein Synthesis in E. coli. II. The Influence of Specific Photoproducts. Proc. Natl. Acad. Sci. US 50, 657 (1963).

3. Grossman, L., Ono, J. and Wilson, R. The Effects of Ultraviolet Light on Nucleic Acid Coding Systems. In "Cellular Radiation Biology", 18th Annual Symp. Fund. Cancer Res., 1964, M.D. Anderson Hospital. Williams and Wilkins, Baltimore, 1965, p. 107.

4. Grossman, L., Ono, J. and Wilson, R.G. Biochemical Significance of Ultraviolet Induced Water Addition to Nucleic Acid Pyrimidines. Federation Proc. 24, S-80-S-84 (1965).

5. Ono, J., Wilson, R.G. and Grossman, L. Effects of Ultraviolet Light on the Template Properties of Polycytidylic Acid. J. Mol. Biol. 11, 600 (1965).

6. Simon, M.I., Grossman, L. and Van Vunakis, H. Photosensitized Reaction of Polyribonucleotides. I. Effect on Their Susceptibility to Enzyme Digestion and Their Ability to Act as Substrate Messengers. J. Mol. Biol. 12, 50 (1965).

7. Phillips, J.H., Brown, D., Adman, R. and Grossman, L. The Effects of Hydroxylamine on Polynucleotide Templates for RNA Polymerase. J. Mol. Biol. 12, 816 (1965).

8. Phillips, J.H., Brown, D., and Grossman, L. The Efficiency of Induction of Mutations by Hydroxylamine. J. Mol. Biol. 21, 405 (1966).

9. Adman, R., and Grossman, L. Template Properties of Polyribonucleotides Containing Uracil or Modified Uracil in the RNA Polymerase Reaction. Appendix: Statistical Characteristics of Template Sites Required for Reiteration. J. Mol. Biol. 23, 417 (1967).

10. Grossman, L. Studies in Mutagenesis Induced in vitro. Photochem. Photobiol. 7, 727 (1968).

11. Grossman, L. and Brown, D.M. The Nature and Influence of Ultra-Violet and Hydroxylamine Lesions in Nucleic Acids and the Enzymatic Repair of the Former. Ciba Symp., Mutation as a Cellular Process (1969).

12. Banks, G.R., Brown, D.M., Streeter, D.G. and Grossman, L. Mutagenic Analogues of Cytosine: RNA Polymerase Template and Substrate Studies. J. Mol. Biol. 60, 425 (1971).

13. Mahler, I. and Grossman, L. Transformation of Radiation Sensitive Strains of Micrococcus lysodeikticus. Biochem. Biophys. Res. Commun. 32, 776 (1968).

14. Kaplan, J.C., Kushner, S.R. and Grossman, L. Enzymatic Incision and Excision of UV-Irradiated DNA. Federation Proc. 28, 2, 465 (1969).

15. Grossman, L., Kaplan, J.C., Kushner, S.R. and Mahler, I. Enzymes Involved in the Early Stages of Repair of Ultraviolet-Irradiated DNA. Cold Spring Harbor Symp. 33, 229 (1968).

16. Grossman, L., Kaplan, J., Kushner, S., and Mahler, I. Enzymatic Mechanisms for the Repair of UV-Irradiated DNA. Ann. 1st Super. Sanita 5, 318 (1969).

17. Kaplan, J.C., Kusner, S.R. and Grossman, L. Enzymatic Repair of DNA. I. Purification of Two Enzymes Involved with Excision of Thymine Dimers from UV-Irradiated DNA. Proc. Natl. Acad. Sci. US 63, 144 (1969).

18. Kaplan, J.C., Kushner, S.R. and Grossman, L. Enzymatic Repair of DNA. III. Properties of the UV-Endonuclease and UV-Exonuclease. Biochemistry 10, 3315 (1971).

19. Kushner, S.R., Kaplan, J.C., Ono, H. and Grossman, L. Enzymatic Repair of Deoxyribonucleic Acid. IV. Mechanism of Photoproduct Excision. Bichemistry 10, 3325 (1971).

20. Mahler, I., Kushner, S.R. and Grossman, L. In vivo Role of the UV-Endonuclease from Micrococcus luteus in the Repair of DNA. Nature New Biology 234, 47 (1971).

21. Grossman, L., Garvik, B.M., Ono, H.K., Braun, A.G., Hamilton, L.D.G. and Mahler, I. Mechanisms of Excision Repair in "DNA Synthesis In vitro" (Wells, R.D. and Inman, R.B., eds.) University Park Press, Baltimore, Maryland, p. 27 (1973).

22. Braun, A. and Grossman, L. Enzymatic Repair of DNA. V. An Escherichia coli UV Specific Endonuclease which is Absent from the uvrA and uvrB Mutants. Proc. Natl. Acad. Sci. 71, 1838 (1974).

23. Hamilton, L., Mahler, I. and Grossman, L. Enzymatic Repair of DNA: The Biochemical and Biological Repair Properties of a DNA Polymerase from Micrococcus luteus. Biochemistry 13, 1886 (1974).

24. Mahler, I., George, J. and Grossman, L. In vitro Repair of UV-Irradiated Micrococcus luteus Bacteriophage N1 Transfecting DNA. J. Virology 13, 765 (1974).

25. Grossman, L. Enzymes Involved in the Repair of DNA. Advances in Radiation Biology, Vol. III, 77 (1974) Review.

26. Grossman, L. Enzymatic Repair of DNA in Bacteria. In Molecular Mechanisms for the Repair of DNA, eds. P.C. Hanawalt, R.B. Setlow, Plenum Press, New York (1974).

27. Grossman, L., Braun, A., Feldberg, R. and Mahler, I. Enzymatic Repair of DNA. Annual Revs. of Biochemistry 44, 19-43 (1975) Review.

28. Grossman, L., Braun, A., Garvik, B., Hamilton, L. and Mahler, I. Enzymes that Repair or Edit Ultraviolet-Irradiated DNA, Novas Tendencias em Fotobiologia, 45, 167 (1975). Supplemento dos Anais da Academia Brasileira de Ciencias.

29. Feldberg, R. and Grossman, L. A DNA Binding Protein from Human Placenta Specific for Ultraviolet Damaged DNA. Biochemistry 15, 2402-2408 (1976).

30. Doniger, J. and Grossman, L. Enzymatic Repair of DNA: Isolation Purification and Properties of a Pyrimidine Dimer Excising Enzyme from Human Placenta. J. Biol. Chem. 251, 4579 (1976).

31. Braun, A. and Grossman, L. Enzymatic Repair of DNA: Site of Action of the E. coli Incising Enzyme. Biochemistry 15, 416 (1976).

32. Riazuddin, S. and Grossman, L. Micrococcus luteus Correndonucleases: I. Resolution and Purification of Two Endonucleases Specific for DNA Containing Pyrimidine Dimers. J. Biol. Chem. 252, 6280 (1977).

33. Riazuddin, S. and Grossman, L. Micrococcus luteus Correndonuclease: II. Mechanism of Action of Two Endonucleases Specific for DNA Containing Pyrimidine Dimers. J. Biol. Chem. 252, 6287 (1977).

34. Riazuddin, S., Grossman, L. and Mahler, I. Micrococcus luteus Correndonuclease: III. Evidence for Involvement in Repair in vivo of Two Endonucleases Specific for DNA Containing Pyrimidine Dimers. J. Biol. Chem. 252, 6294 (1977).

35. Grossman, L., Riazuddin, S., Doniger, J. and Hamilton, L. Enzymes Involved in the Repair of Damaged DNA. NATO Summer Course, "DNA Synthesis-Present and Future", Sicily (1977) in press.

36. Grossman, L., Riazuddin, S., K. Lindahl and W. Haseltine. Nucleotide Excision Repair of Damaged DNA. Cold Spring Harbor Symposium on DNA; Replication and Recombination, 1978.

37. Chen, G. and Grossman, L. The Facilitative Effects of the E. coli DNA Binding Protein on Excision of Damaged Nucleotides.

38. Hamilton, L., Pedrini, A.M. and Grossman, L. J. of Supramolecular Structure Supp. 2, 54 (1978).

39. Shaper, L.N., and Grossman, L. Isolation and Characterization of the Ap Endonuclease from Human Placenta. ICN-UCLA Symposium on DNA Repair Mechanisms, Colorado, 1978.