Ann. Ist. Super. Sanità
Vol. 25, N. 1 (1989), pp. 191-202

PROCESSING OF RING SATURATION AND FRAGMENTATION PRODUCTS OF DNA THYMINE IN *ESCHERICHIA COLI*

S.S. WALLACE

Department of Microbiology, University of Vermont, Burlington, Vt, USA

Summary. - *The biological processing of thymine ring saturation and fragmentation products is summarized in Table 1. The ring saturation product, thymine glycol, is a block to* in vitro *DNA synthesis, whereas the ring saturation product, dihydrothymine, is not. Both these lesions are recognized* in vitro *by endonucleases III and VIII. Since thymine glycol is a replicative block, it is a lethal lesion* in vivo. *The excision repair process for removal of thymine glycols from DNA is initiated* in vivo *by endonuclease III and is followed by the action of either exonuclease III or endonuclease IV. Thymine glycol is very efficiently bypassed by translesion bypass in both single and double stranded DNA, however, because thymine glycol templates an adenine (A) and retains pairing characteristics, it is at best a weakly mutagenic lesion. The thymine ring fragmentation product, urea, and apurinic/apyrimidinic (AP) sites are both strong blocks to* in vitro *DNA synthesis. Both are substrates* in vitro *for endonucleases III, IV, VIII and IX as well as exonuclease III. Both are lethal lesions in single stranded and double stranded phage transfecting DNA. The excision repair of urea residues and AP sites is initiated* in vivo *by either exonuclease III or endonuclease IV. Neither of these noninstructive lesions are efficiently bypassed by UV-induced translesion bypass, however, when bypass occurs mutations result. β-ureidoisobutylic acid is also a block to DNA synthesis* in vitro. *DNA containing this lesion is a substrate for endonucleases VIII and IX. The biological processing of this ring open thymine fragmentation product has yet to be determined. Thus, these ring saturation and fragmentation products of thymine have provided a point of departure for understanding the biological processing of modified bases with altered pairing and/or stacking properties.*

Riassunto (Processi biologici che intervengono sui prodotti di frammentazione e saturazione della timina del DNA in *Escherichia coli*). - *Il processamento biologico dei prodotti di saturazione e frammentazione dell'anello della timina è riassunto nella Tab. 1. Il prodotto di saturazione dell'anello, glicole della timina, costituisce un blocco per la sintesi del DNA* in vitro, *mentre non lo è un altro prodotto di saturazione, la diidrotimina. Entrambe le lesioni sono riconosciute* in vitro *dalle endonucleasi III e VIII. Il glicole della timina essendo un blocco della replicazione, è una lesione letale* in vivo. *Il processo di riparazione per excisione* in vivo *dei glicoli della timina dal DNA inizia con l'intervento della endonucleasi III seguito dall'azione della esonucleasi III o dall'endonucleasi IV. Il glicole della timina è molto efficientemente oltrepassato dal meccanismo di "bypass translesione" in DNA a singolo e doppio filamento. Tuttavia poiché il glicole della timina fa da stampo per una adenina mantenendo la caratteristiche di accoppiamento corretto, è al massimo una lesione debolmente mutagena. Il prodotto di frammentazione dell'anello della timina, l'urea, e i siti apurinici/apirimidinici (AP) sono entrambi forti blocchi per la sintesi del DNA* in vitro. *Entrambe queste lesioni sono substrati* in vitro *sia per le endonucleasi III, IV, VIII e IX che per la esonucleasi III. Entrambe sono lesioni letali nel DNA di fagi trasfettati a singolo o a doppio filamento. La riparazione per excisione dei residui di urea e dei siti AP inizia* in vivo *con l'intervento della esonucleasi III o della endonucleasi IV. Queste lesioni "non informazionali" non sono sufficientemente oltrepassate dal "bypass translesione" indotto dall'UV, tuttavia se questo bypass avviene queste lesioni risultano mutagene. L'acido β–ureidoisobutilico costituisce un blocco per la sintesi del DNA* in vitro. *Il DNA contenente questa lesione è un substrato per le endonucleasi VIII e IX. Il processamento biologico di questo prodotto di frammentazione ad anello aperto della timidina non è stato ancora determinato. In conclusione questi prodotti di saturazione e frammentazione dell'anello della timina hanno fornito un punto di partenza per la comprensione del processamento biologico di basi modificate con proprietà alterate di accoppiamento e/o inserimento.*

Modified DNA bases as models for free radical-induced DNA damage

Free radicals produce a broad spectrum of damage in DNA thus making it difficult, if not impossible, to assess the biological consequences of individual damages. In order to circumvent this problem, we have employed the strategy of selectively introducing unique lesions of interest into viral DNA molecules, so that we can determine if they are substrates for repair enzymes and assess their biological processing [1, 2]. We have compared the properties of four modified thymine residues (Fig. 1), thymine glycol, urea, dihydrothymine, and β-ureidoisobutylic acid, to sites of base loss (apurinic/apyrimidinic, AP sites). Thymine glycol is a model for thymine ring saturation products and has been found in DNA X-irradiated *in vitro* [3-5] and *in vivo* [6, 7]. Thymine glycols are also believed to be formed *in vivo* as a consequence of oxidative stress [8, 9]. Urea residues are stable fragmentation products of thymine hydroperoxides that are found in DNA X-irradiated *in vitro* [3] or oxidized with hydrogen peroxide [10]. Dihydrothymine, another ring saturation product of thymine, is produced in DNA by irradiation under anoxic conditions [11]. β-ureidoisobutylic acid is the ring cleavage product of dihydrothymine and is not produced *in vivo*. Apurinic/apyrimidinic (AP) sites are probably the most common lesion encountered by the cell. AP sites are formed by free radical interactions with DNA [12], by variety of chemical agents [13], and are intermediates in excision repair initiated by DNA glycosylases [14]. AP sites are also produced in DNA under normal physiological conditions [15].

Thymine glycol and dihydrothymine are relatively minor alterations of DNA thymine. Both should retain base pairing characteristics, however, since the C5-C6 double bond is saturated, both these compounds are no longer planar and assume a half chair conformation with C5 and C6 significantly out of the plane of the other four ring atoms. Thus these two modified bases are good models for altered pyrimidines that may retain pairing but have altered stacking characteristics. In contrast, urea residues and AP sites are totally noninstructive lesions; in the case of AP sites, there is no base attached to the deoxyribose, and in the case of urea residues, a urea fragment is attached. Thus these compounds are good models for base damages that retain no pairing or stacking characteristics. β-ureidoisobutylic acid should retain no pairing characteristics but might be able to still form stacking interactions.

Introduction and quantitation of modified DNA bases

Thymine glycols can be selectively introduced into DNA by osmium tetroxide oxidation. In DNA, thymine residues are oxidized in a ratio of 99 to 1 cytosine

Table 1. - *Processing of unique DNA lesions in* Escherichia coli

Lesion	*In vitro* replicative block	Substrate for *E. coli* repair enzymes	Lethal lesion	Excision repair	SOS lesion bypass	Mutagenic lesion
thymine glycol	yes	endo III endo VIII	yes	endo III, exo III or endo IV	very efficient	no (weak)
dihydrothymine	no	endo III	predicted no	-	-	predicted no
urea residues	yes	endo III exo III endo IV endo VIII endo IX	yes	exo III or endo IV	inefficient	yes
β-ureidoisobutylic acid	yes	endo VIII endo IX	predicted yes	?	?	?
AP sites	yes	endo III exo III endo IV endo VIII endo IX	yes	exo III or endo IV	inefficient	yes

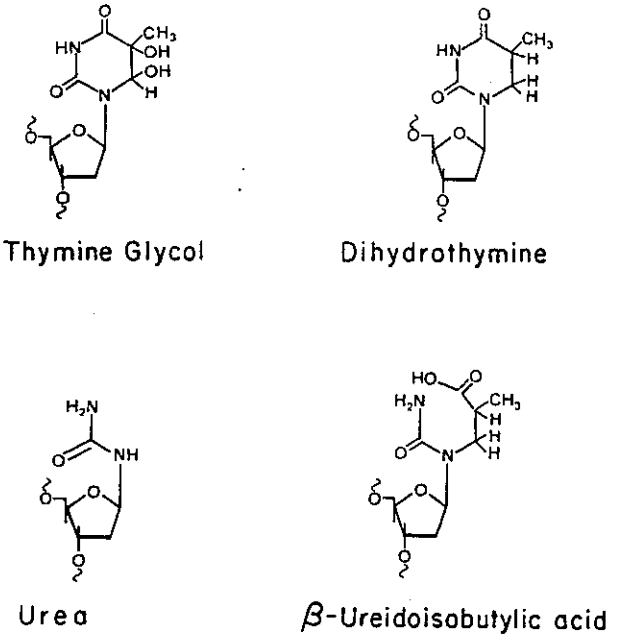

Fig. 1. - Structural formula of thymine glycol, dihydrothymine, urea and β-ureidoisobutylic acid.

residue [16]. Further, of the thymine residues oxidized, approximately 85% of these are cis-thymine glycol [17]. Thus cis-thymine glycol is the predominant but not exclusive [18] product formed in DNA by osmium tetroxide oxidation. The ability to selectively produce thymine glycol in DNA provides the opportunity to determine the consequences of this lesion in the absence of other oxidation products. Thymine glycol can be quantitated by a chemical assay that relies on the recovery of the alkali cleavage product of thymine glycol, an acetol fragment, that contains the methyl group of thymine which was previously radioactively labeled [19]. Although this method was in widespread use for a number of years, it suffers from a relative lack of specificity and sensitivity. Thymine glycols can be relatively easily quantitated at low levels of damage in supercoiled DNA by measuring enzyme-induced cleavage of thymine glycol-containing DNA by enzymes, such as endonuclease III of *Escherichia coli (E. coli)* that specifically recognize the damage [20, 21]. The cleavage of supercoiled DNA produces the open circular form; these two forms can be readily separated from one another by either agarose gel electrophoresis or fluorometry [22] thus providing a relatively simple way to assay this lesion. Thymine glycols can also be easily quantitated by using immunochemical techniques [4, 6, 21]. We have elicited antibodies to thymine glycol by chemically synthesizing thymidine glycol monophosphate, conjugating it to protein carrier, and then immunizing rabbits [4] or producing mouse monoclonal antibodies (B. Erlanger, unpublished observations). The antibody assay has the advantage of being highly specific and extremely sensitive, detecting femtomole levels of damage in DNA [4, 21].

DNA containing urea residues is formed by alkali treatment of osmium tetroxide-oxidized DNA containing thymine glycol residues. Alkali treatment quantitatively converts thymine glycol into urea residues [10]. Urea residues can be easily measured in DNA because they are susceptible to the action of a number of *E. coli* endonucleases, including endonucleases III [23] and IV [24], as well as the endonuclease activity of exonuclease III [10]. The conversion of thymine glycols to urea residues is simply monitored by the production of exonuclease III-sensitive sites from exonuclease III-resistant sites. Conversion can also be monitored by the loss of immunochemical signal to anti-thymine glycol antibody.

Dihydrothymidine triphosphate can serve as a substrate *in vitro* for DNA polymerase I of *E. coli* [25]. Sequencing gel analysis has demonstrated that dihydrothymidine triphosphate only substitutes for T in this reaction (Ide and Wallace, unpublished observations). This provides a useful way for introducing this modified base into DNA, that is, by a nick translation reaction using radioactively labeled dihydrothymidine triphosphate. Interestingly, dihydrothymidine triphosphate is not a substrate for the T4 or avian myoblastosis polymerases. The reason for this appears to be that dihydrothymidine triphosphate does not bind to the substrate binding site of these latter two enzymes. Dihydrothymine has also been introduced into phage DNA by supplying the nucleoside form to phage-infected cells [26]. Dihydrothymine in DNA can be quantitated either by its ability to serve as a substrate for certain enzymes, such as *E. coli* endonuclease III [27], or by an immunochemical reaction with an anti-dihydrothymine antibody [5]. In the former case, the enzyme-induced nicking can be measured by the conversion of supercoiled to relaxed DNA as described above. In the latter case, a polyclonal antibody elicited to dihydrothymidine monophosphate conjugated to a protein carrier is used. Again this antibody is highly specific and very sensitive.

When DNA containing dihydrothymine is hydrolyzed in alkali, the dihydrothymine cleavage product, β-ureidoisobutylic acid results. Although this product is not produced *in vivo*, it is useful as a model for ring-open pyrimidine products. β-ureidoisobutylic acid can also be quantitated by its susceptibility to repair endonucleases such as endonuclease VIII of *E. coli* [24].

Apurinic sites are readily formed by heating DNA at acid pH [28]. In this case, guanine is selectively released over adenine. Apurinic sites can be easily quantitated by their susceptibility to β-elimination cleavage in alkali or by their susceptibility to a variety of apurinic endonucleases [20, 21]. The resulting strand break can be then assayed by the techniques described above.

The ability of modified DNA bases to act as blocks to DNA synthesis *in vitro*

One way to predict the possible biological consequences of unique modified bases is to evaluate their interaction with DNA polymerases *in vitro*. The DNA

base of interest is modified on a single stranded template which is then annealed to oligonucleotide primer. This template-primer is then used in a polymerization reaction with *E. coli* DNA polymerase I or other DNA polymerases of interest. The newly synthesized extended primer is then examined on a DNA sequencing gel to determine if and where the modified base on the template strand blocked polymerization of the newly synthesized strand [29, 30].

Since thymine glycol and dihydrothymine are relatively minor alterations of thymine, that is, base pairing characteristics might be preserved whereas stacking characteristics are altered, it was of interest to assess whether they may be blocks to polymerization *in vitro*. To do this, single stranded M13 DNA was oxidized with osmium tetroxide [31-33] or potassium permangenate [34] to produce thymine glycol, and the ability of this strand to act as template for DNA polymerase I examined. The results showed that thymine glycols are very efficient blocks to polymerization by both *E. coli* DNA polymerase I as well as T4 DNA polymerase. Interestingly, polymerization was arrested opposite the putative thymine glycol lesion with an A being incorporated [35]. Thus thymine glycols do retain pairing characteristics as measured by this *in vitro* assay, however, the alteration of the pyrimidine ring is such that chain extension is inefficient and is influenced by the sequence context [31, 32, 35].

A similar experiment was done with f1 DNA containing dihydrothymine in the template strand (Ide and Wallace, unpublished observation). In this case, dihydrothymine monophosphate was incorporated into single stranded f1 DNA *in vivo* using dihydrothymidine as a substrate. Dihydrothymine was only a weak block to DNA polymerase I *in vitro*. This is not surprising since dihydrothymidine triphosphate was an effective substrate for DNA polymerase I *in vitro* [25]. It is interesting to note that the substitution of two hydroxyl groups in the case of thymine glycol for two hydrogens in the case of dihydrothymine can determine whether or not the modified base can serve as substrate for or act as a block to DNA polymerase.

These *in vitro* results suggest that thymine glycol should be a lethal lesion whereas dihydrothymine should not or at best be a weak one. Further, since A is incorporated opposite the putative thymine glycol in the template strand, thymine glycols should not be strong premutagenic lesions once the block to replication is relieved by inducing the cellular SOS response. Although it has not been unequivocally established that dihydrothymine templates an A, it only substitutes for T in an incorporation reaction (Ide and Wallace, unpublished observations). Thus dihydrothymine should not be a mutagenic lesion.

When single stranded M13 DNA containing thymine glycols is alkali hydrolyzed to produce urea residues and the primer-template complex is used in an *in vitro* polymerization reaction, urea residues were found to be strong blocks to DNA polymerase I [31, 32]. However, in this case, synthesis was arrested at one base prior to the putative urea residue. These results are similar to those obtained with the totally noninstructive AP site [36]. The fact that urea residues are blocks to DNA replication *in vitro* predicts that they would be lethal lesions *in vivo*. Furthermore, since urea residues appear to have no pairing capacity, if the block to replication is relieved by SOS induction of the cells, urea residues should be mutagenic lesions.

When a similar experiment was done with the ring cleavage product of dihydrothymine, β-ureidoisobutylic acid, blocks to replication were observed (Ide and Wallace, unpublished observations). However in this case, as was observed with thymine glycol, a base, as yet undetermined, was inserted opposite β-ureidoisobutylic acid. This was a surprising result in light of the fact that this ring open fragmentation product would not be expected to maintain its base pairing characteristics. Thus β-ureidoisobutylic acid is predicted to be a lethal and possibly a mutagenic lesion.

In summary, the *in vitro* behavior with DNA polymerases of the modified thymine residues examined gave four different results depending on the stacking and pairing characteristics of the particular modified base. Thymine glycol, urea, and β-ureidoisobutylic acid are all strong blocks to *in vitro* DNA polymerization with synthesis being arrested opposite thymine glycol and β-ureidoisobutylic acid residues and one base before urea residues. Dihydrothymine is not a block to DNA synthesis *in vitro*.

E. coli apurinic endonucleases that recognize oxidative DNA base damages

The major apurinic endonuclease in *E. coli* is exonuclease III. Exonuclease III, originally identified as 3' to 5' exonuclease and phosphatase activities on duplex DNA [37], accounts for about 90% of the apurinic activity [38] that nicks 5' to the apurinic site (class II apurinic endonuclease) [39]. Not only does the apurinic activity of exonuclease III incise 5' to apurinic sites, but this activity also incises DNA containing urea fragmentation products [10], as well as a series of alkoxyamine-modified derivatives of apurinic sites [40]. Exonuclease III also has diesterase activity against 3' phosphoglycoaldehyde esters in model DNA substrates containing these residues [41]. Thus exonuclease III has an endonucleolytic activity that is capable of incising adjacent to a number of fragmented products in addition to AP sites. Endonuclease IV represents about 10% of the class II AP activity in *E. coli* [38]; endonuclease IV has no associated exonuclease activity but otherwise recognizes the same broad range of substrates recognized by exonuclease III [24]. DNA containing thymine glycol,

dihydrothymine or β-ureidoisobutylic acid is not a substrate for exonuclease III or endonuclease IV [24].

Endonuclease III is a class I AP endonuclease that incises on the 3' side of an apurinic site [39]. Endonuclease III has an associated glycosylase activity that recognizes a broad spectrum of pyrimidine ring saturation and fragmentation products, including thymine glycol, dihydrothymine and urea residues, [23, 27, 42] and appears to be the principal enzyme in *E. coli* that recognizes these lesions. An enzyme similar to endonuclease III, endonuclease VIII, has recently been identified and purified [2, 43]. This activity also has a 3' AP endonuclease and an associated glycosylase that recognizes a broad spectrum of pyrimidine saturation and fragmentation products that overlaps, but is not a identical, to that of endonuclease III. For example, DNA containing β-ureidoisobutylic acid is a good substrate for endonuclease VIII but not for endonuclease III. Using a thymine glycol-containing substrate, endonuclease VIII represents 5 to 10% of the constitutive activity of endonuclease III. Thus it is possible that endonuclease VIII is regulated and/or that its primary substrate has not been identified. Another novel *E. coli* endonuclease, endonuclease IX, has recently been identified [2, 44]. Like endonucleases III and VIII, endonuclease IX also has a 3' AP endonuclease activity with an associated glycosylase activity. However, the substrate specificity of the glycosylase activity of endonuclease IX thus far seems to be less broad than that of endonucleases III and VIII. Endonuclease IX has been shown to release urea by its N-glycosylic action, however, DNA containing thymine glycol or dihydrothymine is not a substrate for the enzyme. The activity of endonuclease IX on a DNA substrate containing urea residues is only 1-2% that of endonuclease III.

In order to prepare the appropriate substrate for repair polymerization *in vitro*, thymine glycol can be removed from DNA containing this lesion by a sequence of reactions. First, the N-glycosylic action of either endonuclease III or endonuclease VIII removes thymine glycol as a free base, leaving an AP site in DNA. In a concerted reaction [45], the phosphodiester backbone is then cleaved 3' to the AP site left by the N-glycosylic action of endonucleases III and/or VIII. This substrate now contains a 2'3' didehydrodeoxyribose residue on the 3' end of the nicked substrate [46, 47]; this is not an appropriate primer terminus for DNA polymerases and must be removed by the action of a 5' (class II) AP endonuclease, such as exonuclease III or endonuclease IV [39]. With this accomplished, the DNA substrate is now prepared for subsequent repolymerization and ligation to finish the excision repair reaction. Similarly, AP sites and urea residues can be prepared for excision repair synthesis by the action of class II AP endonuclease such as exonuclease III or endonuclease IV.

Biological processing of modified DNA bases: base damages as lethal lesions

The inactivation efficiency of thymine ring saturation and fragmentation products can be determined by introducing these lesions into phage DNA *in vitro* and then assessing its biological activity in a transfection assay. When single-stranded φX [48], M13 [49] or f1 [49] DNA was oxidized with osmium tetroxide to produce thymine glycol, transfected into *E. coli*, and the number of transfectants measured as a function of the number of thymine glycols present in DNA, it took between 1 and 2 thymine glycol lesions to produce an inactivating event. In contrast, in repair proficient hosts, it took between 7-8 thymine glycols to produce an inactivating event in double stranded PM2 transfecting DNA [50] and 11-12 thymine glycols to produce an inactivating event in φX RF DNA [51]. Thus, as predicted from the *in vitro* DNA synthesis studies, thymine glycols are lethal lesions in both single and double stranded viral transfecting DNA.

Similar studies have been done with single stranded DNA containing the noninstructive lesions, AP sites [52] and urea residues (Petrullo and Wallace, unpublished observations). Again, as predicted from the *in vitro* studies, it takes approximately 1-2 of either of these damages to inactivate phage transfecting DNA. When duplex φX RF DNA containing either urea residues or AP sites was used to transfect excision repair proficient hosts, it took approximately 11-12 of these noninstructive lesions to constitute an inactivating event [51]. It took about 7-8 AP sites to produce a single inactivating event in duplex PM2 DNA [50].

Interestingly, the inactivation efficiency of thymine glycols, urea residues and AP sites in either single or double stranded DNA appears to be about the same in wild type cells. Presumably, the inactivation efficiency measured in single stranded DNA reflects the ability of the lesion to constitute a replicative block whereas the inactivation efficiency of the lesion in duplex DNA in wild type hosts is a measure of the cellular repair efficiency for the lesion. Thus, in spite of the fact that thymine glycols retain coding capability, whereas urea residues and AP sites do not, all three appear to be effective replicative blocks both *in vitro* and *in vivo*. Whether or not dihydrothymine or β-ureidoisobutylic acid, the alkali cleavage product of dihydrothymine, constitute lethal lesions *in vivo* is currently under investigation.

Biological processing of modified DNA bases: excision repair

The isolation of *E. coli* mutants defective in exonuclease III, *xth* [53] and endonucleases III, *nth* [54], and IV, *nfo* [55], have allowed for the elucidation of the

excision repair pathway responsible for the lesions recognized by these enzymes. *Nth* mutants, defective in endonuclease III, exhibit no apparent phenotype, that is, they are not sensitive to agents that produce pyrimidine ring saturation and fragmentation products [54], the products recognized by the enzyme. Similarly, single mutants defective in either exonuclease III or endonuclease IV are not particularly sensitive to alkylating agents or X rays that produce AP sites in DNA [55, 56]. However, *xth* mutants are extremely sensitive to hydrogen peroxide [57] and somewhat sensitive to tertbutylhydroperoxide [55] and *nfo* mutants are sensitive to bleomycin and more sensitive than *xth* mutants to tertbutylhydroperoxide [55]. These results suggest that endonuclease IV might recognize some lesions that are not recognized by exonuclease III. Double mutants, *xth nfo*, are sensitive to gamma rays and alkylating agents that produce AP sites and strand breaks in DNA [55].

It was of initial interest to determine whether endonucleases III and IV and exonuclease III were capable of repairing *in vivo* the lesions they recognized *in vitro*. In order to address this question [51], ϕX RF DNA was oxidized with osmium tetroxide to produce thymine glycols and then transfected into either wild type hosts or hosts defective in endonuclease III. The inactivation of ϕX RF DNA by thymine glycols is 2- to 3-fold greater in *nth* mutants than in wild type hosts suggesting that endonuclease III is involved in the repair of thymine glycols *in vivo*. The *in vitro* studies also suggested that an endonuclease III-induced nick on a thymine glycol-containing DNA substrate needs to be acted upon by a class II AP endonuclease before repair polymerization can take place. Accordingly, the inactivation of ϕX RF DNA containing thymine glycols was examined in *xth* mutants, defective in the major class II AP endonuclease in *E. coli*, exonuclease III, and in *nfo* mutants defective in the other class II AP endonuclease, endonuclease IV. No differences in inactivation were observed between wild type or either single mutant host. However, when the inactivation of ϕX RF DNA containing thymine glycols was measured in double mutants defective in both exonuclease III and endonuclease IV, the sensitivity was 2- to 3-fold greater than in wild type. These data suggest that *in vivo*, a class II AP endonuclease is the second step in the excision repair process for a ring saturation product such as thymine glycol and that in *E. coli*, at the levels of damage investigated, either exonuclease III or endonuclease IV can suffice.

Urea residues or AP sites can be directly prepared for polymerization *in vitro* by the action of a class II AP endonuclease, such as exonuclease III or endonuclease IV. In order to test whether this was the first step in the excision repair process of these lesions *in vivo* [51], ϕX RF DNA was treated with osmium tetroxide and alkali-hydrolyzed to produce urea residues, or heated at low pH to produce apurinic sites, and then transfected into *xth* or *nfo* mutants, or *xth nfo* double mutants lacking both exonuclease III and endonuclease IV. The survival of transfecting DNA containing either urea residues or AP sites is the same in either single mutant as in the wild type host, however, the sensitivity in the double mutant, *xth nfo*, is two to three fold greater than wild type. These data suggest that a class II AP endonuclease, either exonuclease III or endonuclease IV, is required for the first step in the *in vivo* excision repair process of noninstructive lesions such as AP sites or urea residues that can be incised *in vitro* by these enzymes.

Interestingly, even in repair deficient hosts, it took between 4-5 lesions to inactivate duplex ϕX DNA. These data suggest either that thymine glycols, urea residues and AP sites are less effective blocks to DNA replication in duplex than in single stranded DNA and/or alternative repair mechanisms (enzymes), such as endonucleases VIII or IX might be functioning. The resolution of this question awaits the isolation of mutants defective in these activities.

Biological processing of modified DNA bases: translesion bypass

ϕX RF DNA containing thymine glycol is activated at a greater rate in mutants lacking endonuclease III, yet this same mutant exhibits no apparent phenotype, that is, *nth* mutants are not sensitive to X rays or other agents that produce thymine glycol and similar products in DNA [54, 55]. This presents a paradox, since it is known that in simple phage systems, stable base damages, the products presumably recognized by endonuclease III, constitute the major proportion of lethal events due to ionizing radiation [50, 58, 59]. Further, it has been shown that thymine ring saturation products are produced in *E. coli* at biologically significant doses [60]. Taken together, these data suggest that either the lesions produced in cellular DNA by ionizing radiation and similar agents do not constitute a significant fraction of the lethal lesions and/or that alternative mechanisms exist for their repair. The resolution of the first alternative awaits the development of sensitive and specific assays to measure the production of individual lesions in radiation-treated or oxidized cellular DNA. There are several potential alternative repair mechanisms. For example, enzymes such as endonucleases VIII and IX might be functioning in a regulated excision repair process, or recombination or translesion bypass might be operational. With respect to the latter, treatment of cells by ionizing radiation and other damaging agents that produce ring saturation and fragmentation products also induces the SOS response [61]. The SOS response includes not only the induction of a number of gene products specifically involved in repair of bulky DNA damages, but also includes a translesion bypass mechanism whereby the specificity of the polymerase is

relaxed so that it now reads through the lesion. In *E. coli*, translesion synthesis requires RecA as well as UmuC and D proteins [62, 63]. If translesion bypass is an efficient repair mechanism for lesions such as thymine glycol, it might be a reason why *nth* mutants are not sensitive to agents that turn on the SOS response, such as X rays. One would expect an *nth* phenotype in the ϕX RF transfecting system however, because damaged viral transfecting DNA does not turn on the SOS system [61].

In support of this notion, when single stranded ϕX [64], f1 [49] or M13 [49] DNA containing thymine glycols is transfected into either uninduced or UV-induced wild type hosts, survival is greatly enhanced in the UV-induced host. Since there is no known mechanism that can repair thymine glycols in single stranded DNA, this increase in survival should represent UV-induced translesion bypass. Similarly, when ϕX duplex DNA containing thymine glycols is transfected into UV-induced hosts, survival is also greatly enhanced compared to uninduced hosts [65]. In fact, when the UV-induced host was lacking the excision repair system for thymine glycols (*nth* mutants), approximately 63% of the lethal lesions are repaired in duplex molecules containing an average of 30 thymine glycols, presumably by UV-induced translesion bypass. Thymine glycols appear to be even more efficiently reactivated by this system than pyrimidine dimers.

In order to determine the specific genetic requirements for translesion bypass of thymine glycols [65], survival of duplex transfecting DNA containing this damage was measured in an *E. coli* strain, *lexA*(Def), that constitutively produces all of the gene products involved in the SOS response [66, 67]. In this strain, even though UmuC and D are produced, no reactivation of ϕX duplex DNA containing thymine glycols was observed unless the cells had been pretreated with UV light. Since treatment by UV light produces damage in DNA and blocks replication it activates the protease function of RecA [61]. Not only does this allow for cleavage of LexA which turns on the SOS response but the activated form of RecA has been shown by Bridges and Woodgate [68-70] to be directly involved in the translesion bypass process for UV damage. In order to determine whether the activated form of RecA was required for translesion bypass of thymine glycols in the *lexA*(Def) strain, a *recA730* mutation was transduced into the *lexA*(Def) strain. *RecA730* mutants have a constitutively activated RecA protease function [71]. In this case, *recA730 lexA*(Def), UV irradiation was not required for reactivation of thymine glycols. Surprisingly, when ϕX RF transfecting DNA containing thymine glycols was transfected into *umuC* mutant hosts, about 50% of the reactivation seen in *umuC*+ hosts was observed. A similar result was found when osmium tetroxide treated single stranded f1 DNA was transfected into *umuC* hosts [49]. *UmuC* mutants show no UV-induced mutagenesis and therefore no UV-induced translesion bypass or reactivation [72]. Reactivation of transfecting DNA containing thymine glycols in umuC mutant hosts is dependent on UV irradiation of the host suggesting that, in addition to SOS translesion bypass, another UV damage-dependent repair mechanism exists for thymine glycols that is independent of the classic SOS response. This was substantiated by the observation that a similar reactivation of ϕX duplex DNA containing thymine glycols occurs in *lexA* and *recA* mutants. However, all SOS-induced reactivation requires the activated form of RecA as well as UmuC. Thus in UV-induced cells, thymine glycols appear to be reactivated either by SOS or by an as yet undefined translesion bypass system. In light of these observations, it is possible that X-ray-induced translesion bypass might be masking a *nth* phenotype for X-ray survival of cells.

In contrast to the observations with thymine glycol-containing DNA, when either single stranded (Petrullo and Wallace, unpublished observations) or duplex [65] DNA containing urea residues or single stranded [52] or duplex [65] DNA containing AP sites was transfected into UV-induced hosts, no increase in survival was observed. These data suggest that noninstructive lesions, such AP sites and urea residues, are not efficiently bypassed by SOS translesion synthesis. It has been suggested that SOS translesion synthesis is a repair process for DNA damage that leads to increased cellular survival at the expense of mutation. This does not appear to be the case for noninstructive lesions such as urea residues and AP sites since no increase in survival is observed. These data suggest that the mutagenesis observed for AP sites and urea residues when SOS processing is induced reflects a low efficiency bypass event.

Biological processing of modified DNA bases: mutagenesis

The mutagenic potential of ring saturation and fragmentation products of thymine can be assessed by a forward mutation assay using either the *E. coli lacZ*′(α) gene contained in M13 hybrid phage DNA. In these experiments the lesion is introduced into the single stranded DNA and the DNA is transfected into appropriate indicator hosts for the determination of the forward mutation frequency. Mutant clones are then isolated and sequenced to determine mutational spectra [73].

Since the *in vitro* DNA synthesis studies using a thymine glycol-containing template DNA indicated that thymine glycol was a pairing lesion, with an A inserted opposite the damage, thymine glycol was not expected to be a mutagenic lesion. However, when M13 or f1 DNA is oxidized with osmium tetroxide to produce thymine glycols, mutations are produced in a dose

dependent manner in both wild type and *umuC* mutants [49]. Further, the mutation frequency is significantly higher in hosts that are pretreated with ultraviolet light. In keeping with the DNA synthesis predictions however, sequence analysis of the hybrid phage mutants showed that mutations occurred preferentially at cytosine rather than a thymine sites. In fact, of the 34 mutants sequenced, 23 of them resulted from C to T transitions. Only one base substitution was at a T site and that was a T to G transversion. Thus thymine glycols do not appear to be a strong premutagenic lesions. In the case of osmium tetroxide oxidation, it appears that a minor cytosine product is responsible for most of the mutations observed. This lesion is probably either uracil glycol or 5-hydroxyuracil. The fact that the number of mutants observed is enhanced in UV-induced hosts probably reflects the induction of translesion bypass repair of lethal thymine glycols, the predominant lesion present, thus allowing for the expression of mutations at the very minor cytosine lesion. Alternatively, or in addition, translesion bypass of uracil glycol might also be required for its mutagenic processing. Essigmann *et al.* [74] have demonstrated, however, that 3 out of 1000 osmium tetroxide-oxidized thymines, probably thymine glycol, inserted site specifically into ϕX single stranded DNA are mutagenic.

The ability of an AP site to constitute a mutagenic lesion has been well studied. Schaaper and Loeb [52], using a reversion assay with depurinated ϕX174 DNA, showed a 20 to 40 fold increase over the spontaneous background of mutations when the SOS response in the host was induced by UV light. Sequence analysis of these revertants showed that adenine residues are preferentially inserted as single base substitutions opposite putative depurinated sites. Kunkel [75], using a forward mutation assay with M13mp2 DNA containing the α segment of the *E. coli lacZ* gene, showed that base substitutions occur opposite AP sites with incorporation of A (59%) greater than T (28%) greater than G (11%) greater than C (1%). These data suggest that A is preferentially incorporated opposite a noninstructive lesion.

When single stranded f1 DNA containing the *lacI* gene was oxidized with osmium tetroxide and alkali hydrolyzed to produce urea residues, mutations were produced in a dose dependent manner (Petrullo and Wallace, unpublished observation). Since these mutants have not yet been sequenced, it cannot be unequivocally determined that they are at sites of urea residues. The fact that the mutation frequency was substantially higher than was observed with osmium tetroxide-treated DNA alone, suggests that urea residues may be mutagenic. Sequencing analysis of these mutants should prove interesting given the preference for A insertion opposite AP sites. If A is inserted preferentially opposite urea residue sites no mutation would result, since the urea residue is derived from a T.

Acknowledgements

I am grateful to my associates both past and present who have been tenacious in their attempt to elucidate the mechanisms involved in the repair of DNA base damage. I am particularly grateful to Drs. Yoke Wah Kow and Robert J. Melamede who are continuing the enzymological studies. We owe the ability to specifically construct both biologically active and substrate DNA molecules containing lesions of interest to the chemical expertise of Dr. Hiroshi Ide. The antibody work has been diligently pursued by Dr. Karen Hubbard in collaboration with Dr. Bernard Erlanger of Columbia University College of Physicians and Surgeons. Lastly, the genetic studies have been expertly carried out by Drs. Michael F. Laspia and Lynn A. Petrullo in my laboratory and in a fruitful collaboration with Dr. J. Eugene LeClerc at the University of Rochester. I am indebted to Kyoung-Jin Shon for help in preparing the manuscript. The work from my laboratory was supported by Public Health Service Grant CA 33657 awarded by the National Cancer Institute and Grant DE-FG02-87-ER60510 from the U.S. Department of Energy.

Review submitted on invitation by the Editorial Board of the *Annali*. Accepted for publication: October 1988.

REFERENCES

1. WALLACE, S.S. 1987. The biological consequences of oxidized DNA bases. *Br. J. Cancer* **55** (Suppl. VIII):118-128.

2. WALLACE, S.S., IDE, H., KOW, Y.W., LASPIA, M.F., MELAMEDE, R.J., PETRULLO, L.A. & LE CLERC, E. 1988. Processing of oxidative DNA base damage in *Escherichia coli*. In.: *Mechanisms and consequences of DNA damage processing*. E.C. Friedberg & P. Hanawalt (Eds). Alan R. Liss, New York. (in press).

3. TEOULE, E., BERT, C. & BONICEL, A. 1977. Thymine fragment damage retained in the DNA polynucleotide chain after gamma-irradiation in aerated solutions. *Radiat. Res.* **72**: 190-200.

4. RAJAGOPALAN, R., MELAMEDE, R.J., LASPIA, M.F., ERLANGER, B.F. & WALLACE, S.S. 1984. Properties of antibodies to thymine glycol, a product of the radiolysis of DNA. *Radiat. Res.* **97**: 499-510.

5. HUBBARD, K., KOW, Y.W., IDE, H. & WALLACE, S.S. 1988. Immunological quantification of thymine radiolysis products in DNA X-irradiated *in vitro*. *J. Cell. Biochem.* (Suppl. 12A); UCLA Symposia on Molecular & Cellular Biology, Abstracts 17th Annual Meetings. Alan R. Liss, Inc. New York. p. 266.

6. LEADON, S.A. & HANAWALT, P.C. 1983. Monoclonal antibody to DNA containing thymine glycol. *Mutat. Res.* **112**:191-200.

7. BREIMER, L.H. & LINDAHL, T. 1985. Thymine lesions produced by ionizing radiation in double-stranded DNA. *Biochemistry* **24**: 4018-4022.

8. CATHCART, R., SCHWIERS, E., SAUL, R.L. & AMES, B.N. 1984. Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. *Proc. Natl. Acad. Sci. USA* **81**: 5633-5637.

9. ADELMAN, R., SAUL, R.L. & AMES, B.N. 1988. Oxidative damage to DNA: Relation to species metabolic rate and life span. *Proc. Natl. Acad. Sci. USA* **85**: 2706-2708.

10. KOW, Y.W. & WALLACE, S.S. 1985. Exonuclease III recognizes urea residues in oxidized DNA. *Proc. Natl. Acad. Sci. USA* **82**: 8354-8358.

11. FURLONG, E.A., JORGENSEN, T.J. & HENNER, W.D. 1986. Production of dihydrothymidine stereoisomers in DNA by gamma-irradiation. *Biochemistry* **25**: 4344-4349.

12. VON SONNTAG, C., HAGEN, U., SCHON-BOPP, A. & SCHULTE-FROHLINDE, D. 1981. Radiation-induced strand breaks in DNA: chemical and enzymatic analysis of end groups and mechanistic aspects. *Adv. Radiat. Biol.* **159**: 109-142.

13. SINGER, B. & GRUNEBERGER, D. 1983. *Molecular biology of mutagens and carcinogens*. Plenum Press, New York. pp. 16-19, 45-96.

14. WEISS, B. & GROSSMAN, L. 1987. Phosphodiesterases involved in deoxyribonucleic acid repair. *Adv. Enzymol.* **60**: 1-34.

15. LINDAHL, T. & NYBERG, B. 1974. Heat-induced deamination of cytosine residues in DNA. *Biochemistry* **3**: 3405-3410.

16. BEER, M., STERN, S., CARMALT, D. & MOHLHENRICH, K.H. 1966. Determination of base sequence in nucleic acids with the electron microscope. V. The thymine specific reactions of osmium tetroxide with deoxyribonucleic acid and components. *Biochemistry* **5**: 2283-2288.

17. FRENKEL, K., GOLDSTEIN, M.S. & TEEBOR, G.W. 1981. Identification of the *cis*-thymine glycol moiety in chemically oxidized and gamma-irradiated deoxyribonucleic acid by high-pressure liquid chromatography analysis. *Biochemistry* **20**: 7566-7571.

18. DIZDAROGLU, M., HOLWITT, E., HAGAN, M.P. & BLAKELY, W.F. 1986. Formation of cytosine glycol and 5,6-dihydroxycytosine in deoxyribonucleic acid on treatment with osmium tetroxide. *Biochem. J.* **235**: 531-536.

19. HARIHARAN, P.V., ACHEY, P.M. & CERUTTI, P.A. 1977. Biological effect of thymine ring saturation in coliphage ϕX-174 DNA. *Radiat. Res.* **69**: 375-378.

20. WALLACE, S.S., KATCHER, H.L. & ARMEL, P.L. 1981. The measurement of X-ray damage in supercoiled DNA by means of enzyme probes. In: *DNA repair: a laboratory manual of research procedures*. E.C. Friedberg & P.C. Hanawalt (Eds). Marcel Dekker, Inc., New York.

21. WALLACE, S.S. 1983. Detection and repair of DNA base damages produced by ionizing radiation. *Environ. Mutagen.* **5**: 769-788.

22. KOWALSKI, D. 1979. A procedure for the quantitation of relaxed closed circular DNA in the presence of superhelical DNA: an improved fluorometric assay for nicking-closing enzyme. *Anal. Biochem.* **93**: 346-354.

23. KATCHER, H.L. & WALLACE, S.S. 1983. Characterization of the *E. coli* X-ray endonuclease, endonuclease III. *Biochemistry*: 4071-4081.

24. KOW, Y.W., IDE, H., MELAMEDE, R.J. & WALLACE, S.S. 1988. Comparative study of the mechanisms of action of the apurinic endonucleases of *E. coli*. *J. Cell. Biochem.* (Suppl. 12A); UCLA Symposia on Molecular & Cellular Biology, Abstracts 17th Annual Meetings. Alan R. Liss, Inc. New York. p. 269.

25. IDE, H., KOW, Y.W., MELAMEDE, R.J. & WALLACE, S.S. 1988. Specificity of DNA N-glycosylase activities of *Escherichia coli* endonucleases III, VII and IX. *J. Cell. Biochem.* (Suppl. 12A); UCLA Symposia on Molecular & Cellular Biology, Abstracts 17th Annual Meetings. Alan R. Liss, Inc. New York. p. 267.

26. IDE, H. & WALLACE S.S. 1988. Dihydrothymine and thymidine glycol 5'-triphosphates as substrates for DNA polymerases: Differential recognition of thymine C5-C6 bond saturation and sequence specificity of incorporation. *Nucleic Acid Res.* (Submitted).

27. DEMPLE, B. & LINN, S. 1980. DNA N-glycosylases and UV repair. *Nature* **287**: 203-208.

28. LINDAHL, T. & ANDERSSON, A. 1972. Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. *Biochemistry* **11**: 3618-3623.

29. MOORE, P.D. & STRAUSS, B.S. 1979. Sites of inhibition of *in vitro* DNA synthesis in carcinogen- and UV-treated ϕX174 DNA. *Nature* **278**: 664-666.

30. SANGER, F., NICKLEN, S. & COULSON, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.

31. IDE, H., KOW, Y.W. & WALLACE, S.S. 1985. Thymine glycols and urea residues in M13 deoxyribonucleic acid constitute replicative blocks *in vitro*. *Nucleic Acids Res.* **13**: 8035-8052.

32. HAYES, R.C. & LE CLERC, J.E. 1986. Sequence dependence for bypass of thymine glycols in DNA by DNA polymerase I. *Nucleic Acids Res.* **14**: 1045-1061.

33. CLARK, J.M. & BEARDSLEY, G.P. 1986. Thymine glycol lesions terminate chain elongation by DNA polymerase *in vitro*. *Nucleic Acids Res.* **14**: 737-749.

34. ROUET, R. & ESSIGMANN, J.M. 1985. Possible role of thymine glycol in the selective inhibition of deoxyribonucleic acid synthesis on oxidized DNA templates. *Cancer Res.* **45**: 6113- 6118.

35. CLARK, J.M. & BEARDSLEY, G.P. 1987. Functional effects of *cis*-thymine glycol lesions on DNA synthesis *in vitro*. *Biochemistry* **26**: 5398-5403.

36. SAGHER, D. & STRAUSS, B. 1983. Insertion of a nucleotide opposite apurinic/apyrimidinic sites in deoxyribonucleic acid during *in vitro* synthesis: uniqueness of adenine nucleotides. *Biochemistry* **22**: 4518-4526.

37. RICHARDSON, C.C., LEHMAN, I.R. & KORNBERG, A. 1964. A DNA phosphatase-exonuclease from *E. coli*. II. Characterization of the exonuclease activity. *J. Biol. Chem.* **239**: 251-258.

38. LJUNGQUIST, S., LINDAHL, T. & HOWARD-FLANDERS, P. 1976. Methyl methane sulfonate-sensitive mutant of *E. coli* deficient in an endonuclease specific for apurinic sites in DNA. *J. Bacteriol.* **126**: 646-653.

39. WARNER, H.R., DEMPLE, B.F., DEUTSCH, W.A., KANE, C.M. & LINN, S. 1980. Apurinic/apyrimidinic endonucleases in repair of pyrimidine dimers and other lesions in DNA. *Proc. Natl. Acad. Sci. USA* **77**: 4602-4606.

40. KOW, Y.W., IDE, H. & WALLACE, S.S. 1988. Mechanism of action of exonuclease III from *Escherichia coli*. In: *Anticarcinogenesis and radiation protection*. P. Cerutti, O. Nygaard & M.G. Simic (Eds). Plenum Press, New York. pp. 135-138.

41. DEMPLE, B., JOHNSON, A. & FUNG, D. 1986. Exonuclease III and endonuclease IV remove 3' blocks from DNA synthesis primers in H_2O_2-damaged *E. coli*. *Proc. Natl. Acad. Sci. USA* **83**: 7731-7735.

42. BREIMER, L.H. & LINDAHL, T. 1984. DNA glycosylase activities for thymine residues damaged by ring-saturation, fragmentation, or ring-contraction are functions of endonuclease III in *Escherichia coli*. *J. Biol. Chem.* **259**: 5543-5548.

43. MELAMEDE, R.J., KOW, Y.W. & WALLACE, S.S. 1988. The isolation and preliminary characterization endonuclease VIII from *Escherichia coli*. In.: *Anticarcinogenesis and radiation protection*. P. Cerutti, O. Nygaard & M.G. Simic (Eds). Plenum Press, New York. pp. 139-144.

44. MELAMEDE, R.J., KOW, Y.W. & WALLACE, S.S. 1988. *Escherichia coli* endonuclease VIII and endonuclease IX: substrate specificity and identification of classes of X-ray-induced DNA damages. *J. Cell. Biochem.* (Suppl. 12A); UCLA Symposia on Molecular & Cellular Biology, Abstracts 17th Annual Meetings. Alan R. Liss, Inc. New York. p. 272.

45. KOW, Y.W. & WALLACE, S.S. 1987. Mechanism of action of *E. coli* endonuclease III. *Biochemistry* **26**: 8200-8206.

46. BAILLY, V. & VERLY, W.G. 1987. *Escherichia coli* endonuclease III is not an endonuclease but a a-elimination catalyst. *J. Biochem.* **242**: 565-572.

47. KIM, J. & LINN, S. 1988. The mechanisms of action of *E. coli* endonuclease III and T4 UV endonuclease (endonuclease V) at AP sites. *Nucleic Acids Res.* **16**: 1135-1141.

48. HARIHARAN, P.V., ACHEY, P.M. & CERUTTI, P.A. 1977. Biological effect of thymine ring saturation in coliphage ϕX174 DNA. *Radiat. Res.* **69**: 375-378.

49. HAYES, R.C., PETRULLO, L.A., HUANG, H., WALLACE, S.S. & LE CLERC, J.E. 198. Oxidative damage in DNA: lack of mutagenicity by thymine glycol lesions. *J. Mol. Biol.* **201**: 239-246.

50. MORAN, E. & WALLACE, S.S. 1985. The role of specific DNA base damage in the X-ray-induced inactivation of bacteriophage PM2. *Mutat. Res.* **146**: 229-241.

51. LASPIA, M.F. & WALLACE, S.S. 1988. Excision repair of thymine glycols, urea residues and apurinic sites in *Escherichia coli*. *J. Bacteriol.* **170**: 3359-3336.

52. SCHAAPER, R.M. & LOEB, L.A. 1981. Depurination causes mutations in SOS-induced cells. *Proc. Natl. Acad. Sci. USA* **78**: 1773-1777.

53. MILCAREK, C. & WEISS, B. 1972. Mutants of *E. coli* with altered deoxyribonucleases, I. Isolation and characterization of mutants for exonuclease III. *J. Mol. Biol.* **68**: 303-318.

54. WEISS, B. & CUNNINGHAM, R.P. 1985. Genetic mapping of *nth*, a gene affecting endonuclease III (thymine glycol-DNA glycosylase) in *Escherichia coli* K-12. *J. Bacteriol.* **162**: 607-610.

55. CUNNINGHAM, R.P., SAPORITO, S.M., SPITZER, S.G. & WEISS, B. 1986. Endonuclease IV *(nfo)* mutant of *Escherichia coli*. *J. Bacteriol.* **168**: 1120-1127.

56. YAJKO, D.M. & WEISS, B. 1975. Mutations simultaneously affecting endonuclease II and exonuclease III in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **72**: 688-692.

57. DEMPLE, B. & HALBROOK, J. 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. *Nature* **304**: 466-468.

58. TAYLOR, W.D. & GINOZA, W. 1967. Correlation of X-ray inactivation and strand scission in the replicative form of ϕX174 bacteriophage DNA. *Proc. Natl. Sci. USA* **58**: 1753-1757.

59. VAN DER SCHANS, G.P., BLEICHRODT, J.F. & BLOK, J. 1973. Contributions of various types of damage to inactivation of biologically active double-stranded circular DNA by gamma-radiation. *Int. J. Radiat. Biol.* **23**: 133-150.

60. SWINEHART, J.L. & CERUTTI, P.A. 1975. Gamma-ray-induced thymine damage in the DNA in coliphage ϕX174 and in *E. coli*. *Int. J. Radiat. Biol.* **27**: 83-94.

61. WALKER, G.C. 1984. Mutagenesis and inducible response to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol. Rev.* **48**: 60-93.

62. DEFAIS, M., FAUQUET, P., RADMAN, M. & ERRERA, M. 1971. Ultraviolet reactivation and ultraviolet mutagenesis of lambda in different genetic systems. *Virology* **43**: 495-503.

63. DEFAIS, M. 1983. Role of *E. coli umuC* gene product in the repair of single-strand DNA phage. *Mol. Gen. Genet.* **192**: 509-511.

64. ACHEY, P.M. & WRIGHT, C.F. 1983. Inducible repair of thymine ring saturation damage in ϕX174 DNA. *Radiat. Res.* **93**: 609-612.

65. LASPIA, M.F. & WALLACE, S.S. 1988. SOS processing of unique oxidative DNA damages in *E. coli*. *J. Mol. Biol.* (Submitted)

66. ENNIS, D.G., FISHER, B., EDMESTON, S. & MOUNT, D.W. 1985. Dual role for *Escherichia coli* RecA protein in SOS mutagenesis. *Proc. Natl. Acad. Sci. USA* **82**: 3325-3329.

67. CALSOU, P. & DEFAIS, M. 1985. Weigle reactivation and mutagenesis of bacteriophage lambda in *lexA*(Def) mutants of *E. coli* K12. *Mol. Gen. Genet.* **201**: 329-333.

68. BRIDGES, B.A. & WOODGATE, R. 1984. Mutagenic repair in *Escherichia coli*: X. The *umuC* gene product may be required for replication post pyrimidine dimers but not for the coding error in ultraviolet-induced mutagenesis. *Mol. Gen. Genet.* **196**: 364-366.

69. BRIDGES, B.A. & WOODGATE, R. 1985. Mutagenic repair in *Escherichia coli*: Products of the *recA* gene and of the *umuD* and *umuC* genes act at different steps in UV-induced mutagenesis. *Proc. Natl. Acad. Sci. USA* **82**: 4193-4197.

70. BRIDGES, B.A. & WOODGATE, R. 1985. The two-step model of bacterial UV mutagenesis. *Mutat. Res.* **150**: 133-139.

71. WITKIN, E.M., McCALL, J.O., VOLKERT, M.R. & WERMUNDSEN, I.E. 1982. Constitutive expression of SOS functions and modulation of mutagenesis resulting from resolution of genetic instability at or near the *recA* locus of *Escherichia coli*. *Mol. Gen. Genet.* **185**: 43-50.

72. KATO, T. & SHINOURA, Y. 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Mol. Gen. Genet.* **156**: 121-131.

73. LE CLERC, J.E., ISTOCK, N.L., SARAN, B.R. & ALLEN, R. Jr. 1984. Sequence analysis of ultraviolet-induced mutations in M13lacZ hybrid phage DNA. *J. Mol. Biol.* **180**: 217-237.

74. ESSIGMANN, J.M. 1988. Replication errors induced by DNA adducts. *J. Cell Biochem.* (Suppl. 12A); UCLA Symposia on Molecular & Cellular Biology, Abstracts 17th Annual Meetings. Alan R. Liss, Inc., New York. p. 251.

75. KUNKEL, T.A. 1984. Mutational specificity of depurination. *Proc. Natl. Acad. Sci. USA* **81**: 1494-1498.