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IN VITRO MUTAGENESIS AND SOS REPAIR

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Summary. - *SOS repair in* Escherichia coli (E. coli) *utilizes inducible gene products to "fix" mutations in the genome. It has been supposed that an "error prone" system is induced which makes errors in the course of bypassing lesions. An alternative model has been proposed by Bridges and Woodgate [1] which suggests that it is the elongation step rather than the insertion opposite a damaged site which is critical in SOS mutagenesis. A variety of* in vitro *evidence supports this more recent model. DNA polymerases can be shown to insert bases opposite non-instructional lesions in DNA. It can be demonstrated that it is the elongation rather than the insertion step which is rate limiting in such* in vitro *reactions. An* in vitro *mutagenesis system can be devised using a processive polymerase with a reduced 3'→5' exonuclease activity. This system mimics the mutagenic specificity seen* in vivo.

Riassunto (Mutagenesi *in vitro* e riparazione del DNA di tipo SOS). - *La riparazione del DNA del tipo SOS in* Escherichia coli (E. coli) *utilizza prodotti genici inducibili per "fissare" le mutazioni nel genoma. E' stata ipotizzata l'induzione di un sistema "error prone" che inserisce errori durante il processo "by-pass" delle lesioni. Bridges e Woodgate [1] hanno proposto recentemente un modello alternativo che indica come critico nella mutagenesi SOS il processo di elongazione piuttosto che l'inserimento di basi scorrette di fronte al sito danneggiato. Molte evidenze sperimentali in sistemi* in vitro *sostengono questo modello. E' stato osservato che le DNA polimerasi inseriscono basi di fronte alle lesioni non-informative nel DNA. E' stato dimostrato che il momento dell'elongazione piuttosto che quello dell'inserzione è il fattore limitante in queste reazioni* in vitro. *In questo studio è stato utilizzato un sistema di mutagenesi* in vitro *costituito da polimerasi con una ridotta attività esonucleasica 3'→5'. Questo sistema è in grado di mimare la specificità mutagena osservata* in vivo.

The SOS repair system of *Escherichia coli (E. coli)* has captured the imagination of geneticists who see this inducible series of reactions as a paradigm with which to explain mutational process in a variety of organisms. Treatment of *E. coli* with agents whose common feature appears to be the inhibition of DNA synthesis results in the synthesis of at least 17 gene products [2]. A related process results in the destruction of the lambda bacteriophage repressor with consequent vegetative virus growth. These gene products, at least some of which aid in promoting survival of the organism, are ordinarily repressed. Induction involves the cleavage of a common repressor, the *lexA* gene product as a result of the activation of the *recA* protein. Exactly how induction results in activation of *recA* protein is not known. It is known that free DNA ends are involved. Whether the *recA* protein is itself a specialized protease, or in some way activates inherent protease activity of the *lexA* repressor, remains to be determined [3].

Approximately 20 years ago, it was discovered by Evelyn Witkin [4, 5] that mutations which block the induction of the SOS pathway in *E. coli* make the host mutagen stable, that is, unable to produce mutations after treatment with ultraviolet light and certain other mutagens. The original strains studied by Witkin *(lexA)* were very sensitive to the killing action of ultraviolet light but gave no detectable induced mutations. The argument that mutations were not recovered because of the lethality of the mutagenic treatment was discarded at that time and was finally eliminated by the discovery of two additional genes, *umuC* and *D*. The *lexA* mutations regulate the entire SOS pathway. The genes particularly concerned with mutagenesis, *umuC* and *D*, were discovered by Kato and Shinoura in 1977 [6] and by Steinborn in 1978 [7] and were later shown to consist of two closely linked genes [8, 9]. *UmuC-D* mutants are mutagen stable but are not by themselves particularly sensitive to the lethal effects of ultraviolet light. The mutations pro-

duced by ultraviolet light and other agents in SOS-induced strains are of a variety of types: transitions, transversions and deletions. These mutations are almost invariably at sites determined by (and generally opposite to) the position of putative lesions in the template although not all sites of reaction are sites for mutation [10]. Untargeted mutations are also observed, for example when unirradiated lambda bacteriophage, used to infect an irradiated host, are found to have an increased frequency of mutations. However, as emphasized by Eisenstadt [10], such untargeted mutations are not dependent on *umuC-D*.

The finding that most mutations occur at the site of lesions which block DNA synthesis leads to the following hypothesis for the SOS mutagenic pathway: DNA replication is inhibited by lesions in the template strand so that a base can not be added opposite the lesion. As a result of the induction of the SOS gene products, polymerase activity is altered so that synthesis past the lesion is possible but at the cost of reduced specificity. This reduced specificity is observed as an increase in the mutation frequency.

An earlier hypothesis ascribes the blockage of DNA synthesis at lesions to the operation of the 3'→5' proofreading exonuclease activity [11]. According to this hypothesis, there should be continual turnover at the site of lesions as the polymerase activity of cells inserts nucleotides. Since these nucleotides are inserted opposite lesions they do not form normal base pairs and are therefore removed by the proofreading exonuclease. It is certainly true that the 3'→5' nuclease plays a role in protecting the cell from mutation since *E. coli* mutants deficient in the ε subunit of polymerase III have high spontaneous mutation rates [12]. In a more modern version of the Villani [11] hypothesis, Lu *et al.* [13, 14] suppose that the *recA* protein plays an additional role in SOS repair by binding to lesions and inhibiting the editing function, therefore decreasing fidelity.

The hypothesis outlined above provides a coherent explanation of the data and has served as a paradigm for thinking about mutation processes in all organisms. The question is whether it is correct in detail for *E. coli* and whether the model of an inducible repair process is helpful in understanding the situation in other organisms. It needs to be emphasized that there is a distinction between an inducible repair system involved in recombinational repair as evidenced by the production of a *recA*-like protein and an inducible mutational process dependent on *umuC-D*-like gene products. The first, induction of a recombinational repair process, is fairly common in the bacteria. The latter, induction of a mutational response via *umuC-D* products is relatively rare. Sedgwick and Goodwin [15] found little or no increase in UV-induced mutations in *Salmonella typhimurium*, *Shigella sonnei*, *Klebsiella aerogenes*, *Citrobacter intermedius*, or *Proteus mirabilis* although these bacteria did become mutable on the introduction of the plasmid pKM101. Even within the genus *Escherichia*, only three out of six species were UV-mutable.

Haemophilus influenzae [16, 17] does not have an inducible mutation system although other aspects of inducible repair are present. Balganesh and Setlow [18] conclude that *Haemophilus* can be considered a *umuC* mutant. *Bacillus subtilus* has a complex inducible repair system but seems not to respond by increased mutagenesis, certainly not to the wide range of agents effective with *E. coli* K12 [19]. *Neisseria gonorrhoeae* [20] and *Acinetobacter calcoaceticus* [21] are devoid of inducible error prone repair. The listing is not meant to be exhaustive but rather to indicate that the distribution of the induced mutability response is capricious and not explicable on the basis of a selective advantage of mutagenesis [22, 15]. How is the distribution to be explained? Plasmid pKM101 (nee' R46, R Brighton, TP120; [23]) derives from a clinically isolated strain. At least eight unrelated groups of plasmids contain genes which complement *UmuC-D* [15]. It may be that the incorporation of different *umuC-D* like plasmid borne genes has occurred numerous times in different bacterial species and that their selection is not related to mutagenesis. Whatever the evolutionary mechanism, *umuC-D* functions seem not to be generally required and inducible (recombinational) repair and mutational repair are different processes. The demonstration that radiation or other mutagenic treatments induce a (variety of) protein products in particular organisms may possibly be irrelevant to studies on mutation in those organisms.

Damage inducible products can be identified in yeast [24]. In mammalian cells, it is clear that a variety of proteins are synthesized in greater amount as a result of damage [25]. The heat shock proteins are a particularly well documented class of such proteins [26]. However, there is as yet no evidence for the participation of any of these inducible proteins in the mutation process. Effects of two or three fold have occasionally been observed as a result of the addition of soluble factors produced by damaged cells to a mutational system [27]. However, a variation in enzyme content (or activity) of this order of magnitude is common in mammalian cells as a consequence of changes incident to the operation of the cell cycle [28] and therefore such changes may not demonstrate a specific inducible mutation response.

Some mammalian cells show a surprising indifference to the presence of "blocking" lesions in their DNA. Although UV lesions are clear blocks to DNA synthesis in human cells, mouse cells survive and replicate their DNA in the presence of such lesions. There is no decrease in survival as compared to human cells [29] and no evidence for a peculiar mutation rate. There is also no evidence for the participation of recombinational repair processes to permit bypass of such lesions. Such *in vivo* observations demonstrate that the lesions have been bypassed, but they do not constitute a demonstration of "translesion synthesis". However, they certainly

are what might be expected if mammalian polymerase systems were able to carry out such synthesis. Therefore, the methods used to handle lesions by those particular *E. coli* strains which produce *umuC-D* products and certain mammalian cell systems may differ considerably.

Even in *E. coli* the same lesion may produce similar (frameshift) mutations by both SOS-dependent and independent pathways [30, 31]. Acetyl aminofluorene lesions produce (-2) frameshifts in an SOS dependent manner when imbedded in repetitive sequences and SOS independent (-2) frameshifts when imbedded in specific non-repetitive sequences [31]. How can this be explained and is there any relationship between this observation and the way the SOS system works?

An alternative to the proposed mechanism for mutation as a result of error-prone bypass accounts for such observations and also for a number of *in vitro* and *in vivo* observations which did not fit the previous schemes. This more recent hypothesis is largely due to the work of Bridges *et al.* [1, 32] who showd that it was possible to obtain UV-induced mutations in *E. coli umuC* mutants. These workers UV-irradiated *E. coli*, incubated for four hours and then released the block to DNA synthesis by photoreactivation. The surviving cells had a higher frequency of mutations, clearly induced by the UV treatment. Bridges concluded that the limiting step in the production of mutants was not the insertion of targeted errors but rather the process of DNA chain elongation. The *umuC-D* functions could be understood as functioning in the elongation step which was seen as rate limiting, rather than in the insertion of nucleotides opposite the lesion. This hypothesis suggests that the SOS effect is quantitative rather than qualitative, a view in accordance with the experiments of Tessman [33] and of LeClerc *et al.* [34]. Both laboratories demonstrated that it is possible to obtain UV-induced mutations in the absence of an induced SOS system. The view, that it is the elongation step rather than the insertion step which is affected by the SOS products is also in accordance with a good deal of the *in vitro* evidence and it is this evidence which I would now like to discuss.

The use of several different DNA polymerases in termination experiments permits the conclusion that the block to DNA synthesis resulting from lesions in the template strand is not a function of the 3'→5' exonuclease activity [35] although there is evidence that exonuclease activity can affect the exact site of termination depending on the lesion and the affected sequence in the DNA. These experiments were done by a variation of the Sanger dideoxy termination technique in which lesions in the template strand act as termination sites. Using ultraviolet-induced lesions termination occurs 3' to the first pyrimidine of a putative dimer with either enzymes including a 3'→5' exonuclease (*E. coli* pol III, pol I, T4 DNA polymerase) or deficient in this activity (human lymphoblast pol α, AMV reverse transcriptase). Depending on the lesion and its position in the DNA sequence, termination may also occur opposite an altered base. However, even when incorporation is opposite a lesion, elongation may be absolutely inhibited as in the case of AMV reverse synthetase-catalyzed synthesis on an acetyl aminofluorene-containing template [36] (Fig. 1). Synthesis may also terminate 3' to the lesion when the template contains abasic sites and polymerases with no 3'→5' exonuclease, such as DNA polymerase α or AMV reverse transcriptase, are used [37] (Fig. 2). A distribution of termination sites before and opposite a lesion is often observed. This phenomenon appears to be sequence specific. It depends, in some way not yet understood, on the nature of the surrounding bases and on the particular lesion. The addition of inhibitors of 3'→5' exonuclease activity can result in extension to opposite a lesion by *E. coli* pol I (Kf) under certain conditions [38] (Fig. 3) but even in such cases, the addition to opposite a lesion need not lead to elongation or translesion synthesis [38]. We concluded that proofreading exonuclease activity was not the reason for the termination in DNA synthesis but rather that termination of DNA synthesis provided time for the exonuclease to act [35].

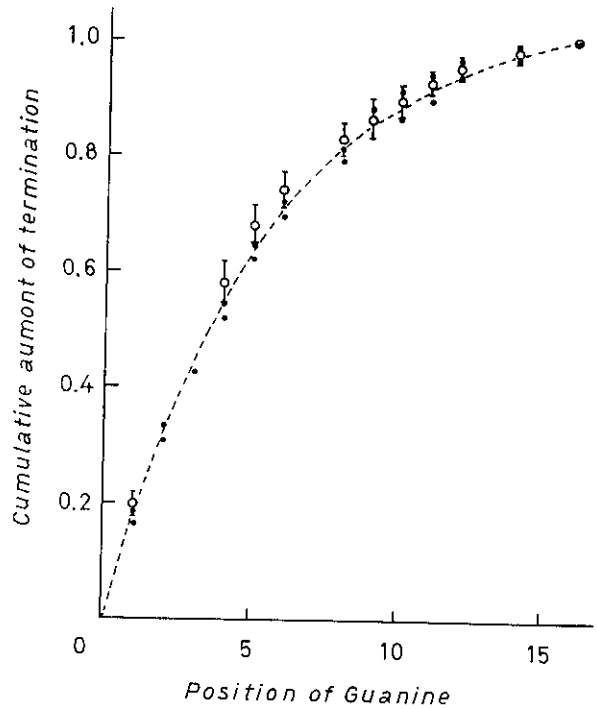


Fig. 1. - Termination by AMV reverse transcriptase on N-acetoxy acetylaminofluorene-treated DNA. Polymerase reactions were carried out on a DNA template containing 210 AAF adducts per ϕX174 molecule. The cumulative amount of termination was plotted as a function of position in a sequence containing 16 guanine sites. Any bypass of adduct would result in data falling below the theoretically calculated, dashed line. The mean and standard error for five experiments with AMV reverse transcriptase (o) and two individual experiments with *E. coli* pol I Kf (●) are shown. (Figure from Moore *et al.* [36], reproduced with kind permission).

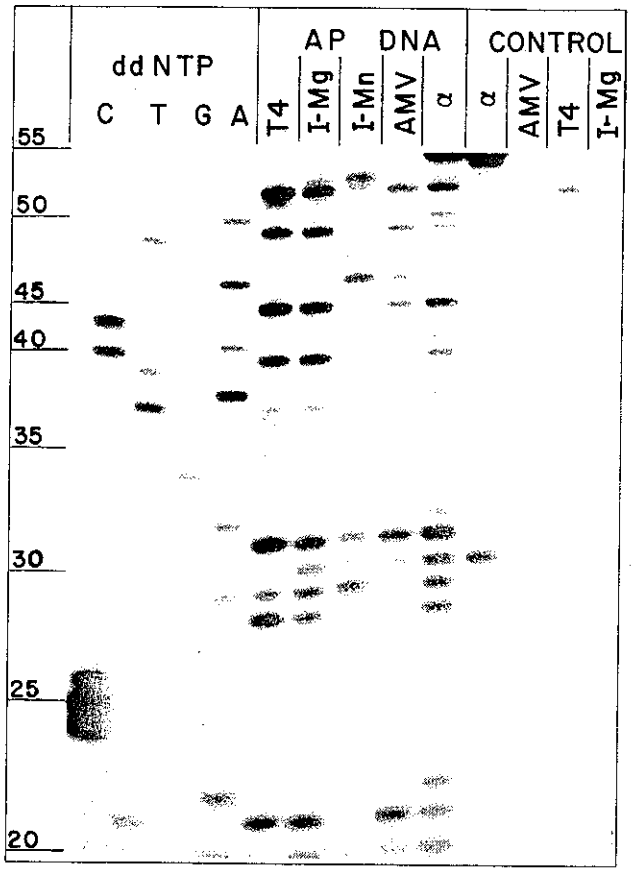


Fig. 2. - Termination of DNA synthesis resulting from abasic sites in DNA. An apyrimidinic template was produced by converting T's to U's by growth of M13 in an *ung⁻ dut⁻* strain and then treating with uracil-N-glycosylase. Control DNA contains U and has not been treated with glycosylase. Termination gel patterns are shown after synthesis with *E. coli* pol I (Kf) with either Mg^{2+} or Mn^{2+}, T4 DNA polymerase, polymerase a (human) or AMV reverse transcriptase. (Figure from Sagher and Strauss [37], reproduced with kind permission).

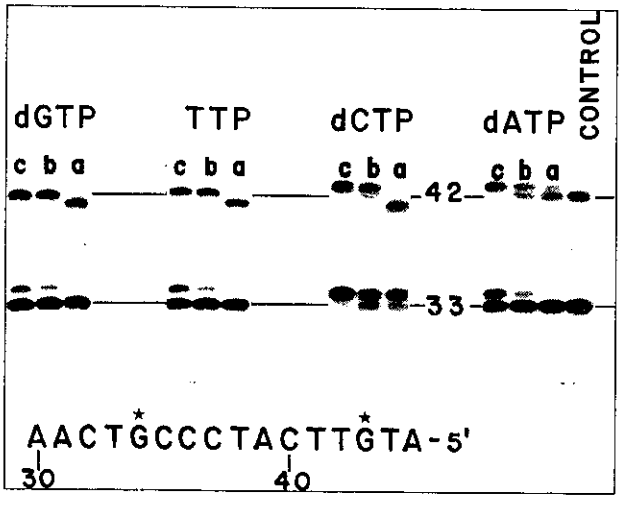


Fig. 3. - Effect of inhibitors of 3'→5' exonuclease activity on nucleotide insertion opposite an acetyl aminofluorene lesion. Newly synthesized DNA terminated 3' to reacted G's in the template (control lane) was incubated in a second stage reaction with the dNTP indicated and (a) no addition; (b) dGMP (14.3 mM); or (c) dAMP (9.3 mM) (Data from Rabkin and Strauss [38]).

Understanding the events at a lesion is made easier by a simple consideration of the kinetics of the process as provided in a paper by Fersht [39] (Fig. 4). The net effect of a lesion on DNA synthesis is determined by the rate of incorporation of a nucleotide opposite the lesion, the rate of removal by proofreading activities and the rate of elongation. If the rate of elongation is slow, then even a weak proofreading activity will suffice to remove a non-pairing nucleotide added opposite a lesion. If the rate of elongation is rapid then the chain can be elongated before proofreading is possible, thereby fixing a mismatched base added opposite a lesion as a mutation. The effect of the "next nucleotide" [39] in increasing mutation frequency can be understood since higher concentrations of the following dNTP(s) will promote faster elongation and a higher error frequency because of the diminished time available for proofreading. This formulation is obviously incomplete since it focuses attention on just the next nucleotide rather than additional following ones and neglects steric factors associated with the lesion and the surrounding sequence. It is nevertheless helpful in understanding the factors involved in replication at a damaged site in DNA.

The *in vitro* results clearly suggest that for many lesions, it is elongation rather than addition opposite a lesion which is rate limiting. That polymerases are able to add nucleotides opposite lesions can be demonstrated by the turnover of dNTP's observed when a partially replicated DNA substrate, with abasic sites terminated 3' to the abasic site, is incubated with dNTP's. With *E. coli* pol I (Kf) as the polymerase, we were able to show a DNA dependent conversion of dNTP→dNMP using a template on which elongation was blocked [37] (Fig. 5). Since the mechanism by which dNTP's are converted to dNMP's involves polymerization followed by excision, this experiment and others in the literature using different enzymes indicates that the polymerases can efficiently add dNTP's to a growing chain even opposite a damaged base. The specificity with which this process occurs is also important as will be discussed below.

Why should it be the elongation step which is limiting? One might suppose that there is inefficient base pairing at the site of addition opposite a damaged base so that the DNA template is not in the correct configuration for further nucleotide addition. This incorrectly configured molecule may therefore be a good substrate for proofreading exonuclease action [39] and in the case of particular substrates where special conditions obtain, permanent termination opposite a lesion may occur. Acetyl aminofluorene adducts to the DNA represent such a special case [36, 38]. DNA polymerases either promote or select rare instances in which the 8-guanyl acetyl aminofluorene adduct is in the informative *trans* configuration making normal base pairing possible (Fig. 6; see also Fig. 3 in which only dC is added at position 33 in the absence of an exonuclease inhibitor). Cytosine is then

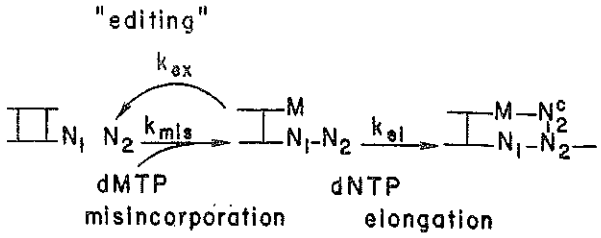


Fig. 4. - Analysis of the factors involved in DNA chain elongation at the site of an altered nucleotide (N1). (Modified from Fersht [39]).

added but, as we interpret it, the adducted guanine swings to the *cis* position in which pairing and elongation is not possible.

Elongation past lesions can be forced without the addition of facilitating proteins. At certain nucleotide sites we [40] synthesized past UV-induced lesions in DNA by carrying out the synthetic reactions in three stages. In the first stage the reaction terminated 3' to a T^T lesion. It has been demonstrated that substitution of Mn^{2+} for Mg^{2+} in polymerase reaction mixtures leads to a relaxation of polymerase specificity. We therefore carried out a "second stage" reaction in the presence of Mn^{2+} and added dA opposite the 5' base of the T^T lesion. A third stage reaction was then performed in which the appropriate dNTP's were provided to permit synthesis to proceed for one or two nucleotides past the 5' T of the lesion. At high concentrations of dNTP and in the presence of Mn^{2+} (and an inhibitor of 3'→5' nuclease action) this bypass synthesis could be demonstrated with *E. coli* pol I (Kf) and either single [40] (Fig. 7) or double stranded [41] DNA templates. Translesion synthesis can therefore be achieved with *E. coli* pol I but only with difficulty and under artificial reaction conditions.

The limiting nature of the elongation step is readily seen in an experiment in which elongation to opposite an acetylaminofluorene-containing dG in the template occurs when dGTP is added in the presence of Mn^{2+} (Fig. 8). Without the addition of nucleotides other than dGTP, elongation occurs to opposite the following (5') C's in the template. As can be seen, accumulation of product opposite the lesion is rapid and this rapid reaction is followed by a slow elongation. We presume that with this particular sequence the ability to rapidly form complementary bases 5' (on the template strand) to the lesion,

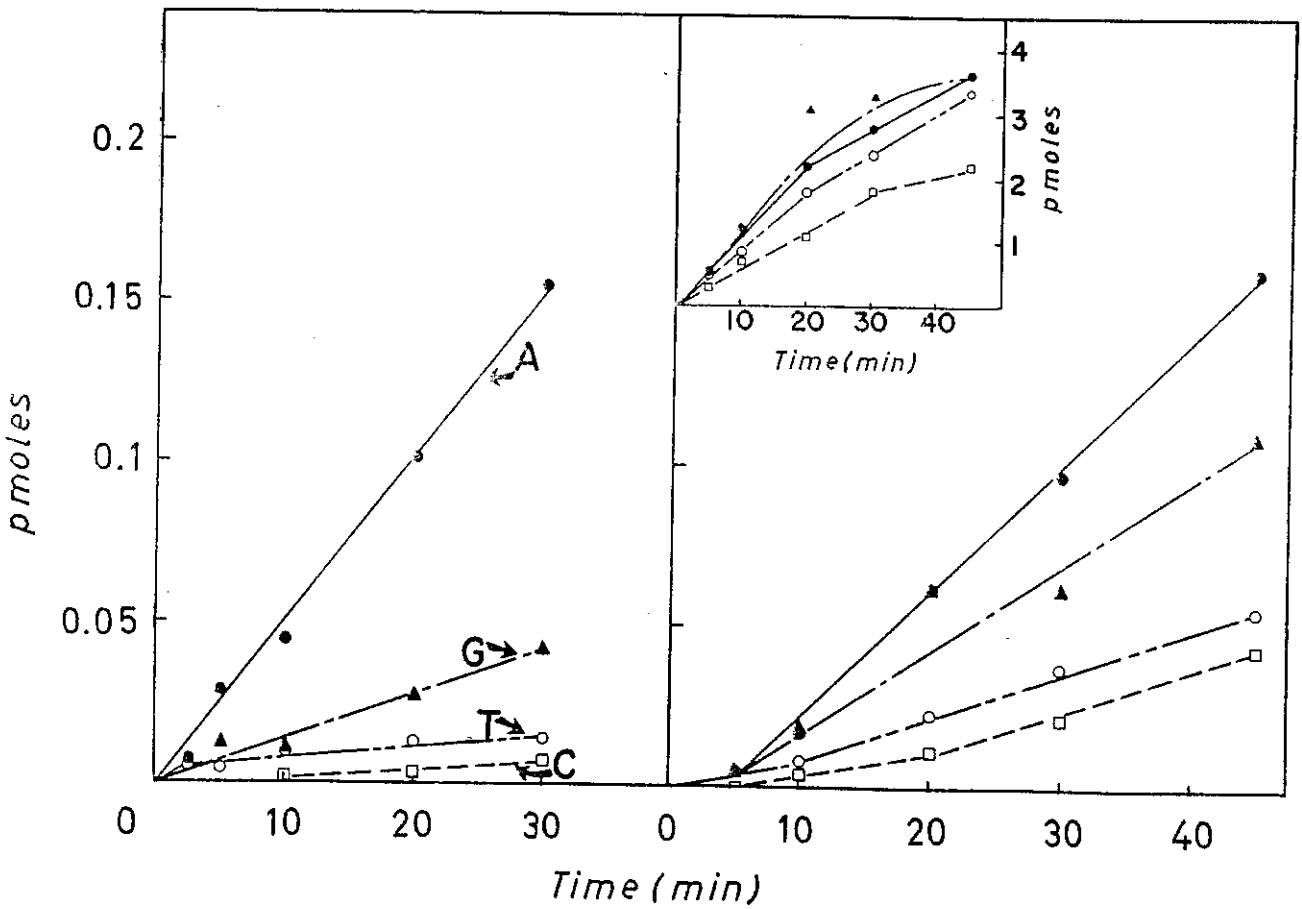
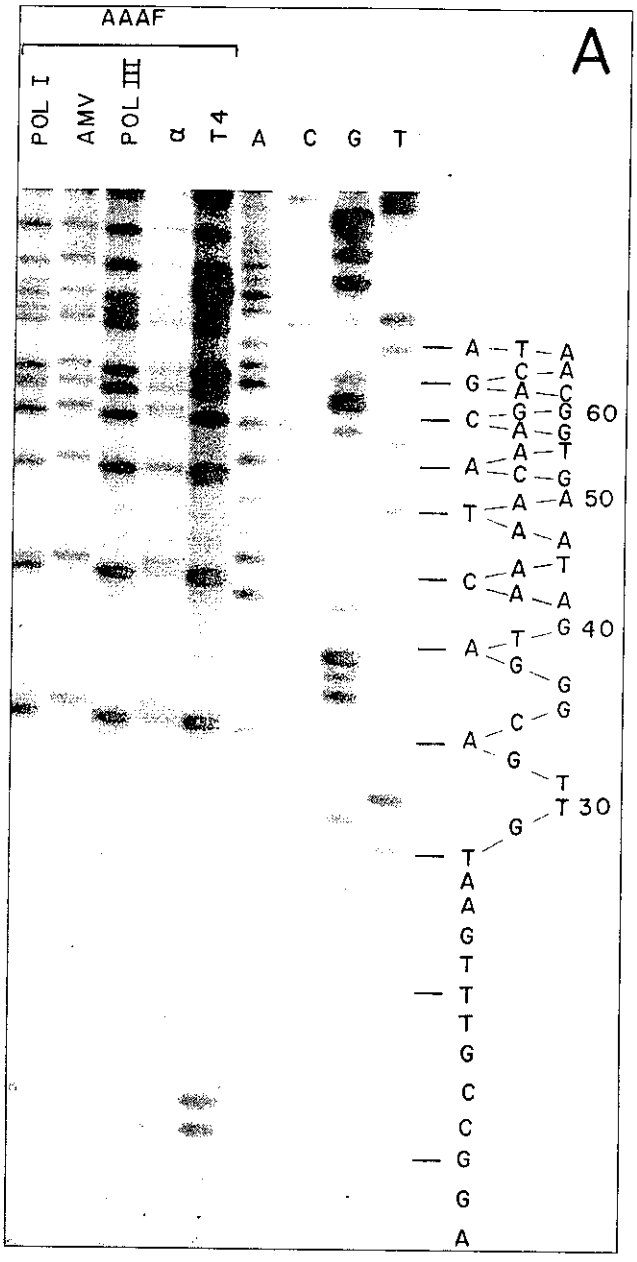
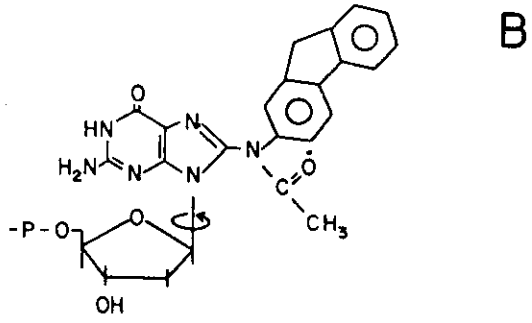


Fig. 5. - *E. coli* pol I (Kf) catalyzed release of dNMP from dNTP in the presence of control or abasic-containing DNA templates. Reaction mixtures contained all four dNTP's and primed template. Left panel: release of dNMP as a result of incubation with an abasic template on which synthesis had terminated prior to abasic sites. Right panel: release of dNMP after incubation with a control DNA template. (Insert: DNA synthesis on the control template). No release of dNMP was detected when either DNA or enzyme was omitted from the reaction mixture. (Figure from Sagher and Strauss, [37], reproduced with kind permission).





syn N-guanin-8-yl-acetyl-2-aminofluorene (AAF-dG)

Fig. 6. - (A) Sequence analysis of products synthesized on an acetyl aminofluorene-containing template by *E. coli* pol I, pol III, T4 DNA polymerase, AMV reverse transcriptase and DNA polymerase a (Daudi). (From Moore *et al.* [35], reproduced with kind permission). (B) The 8-guanyl acetyl aminofluorene adduct in the *cis* position.

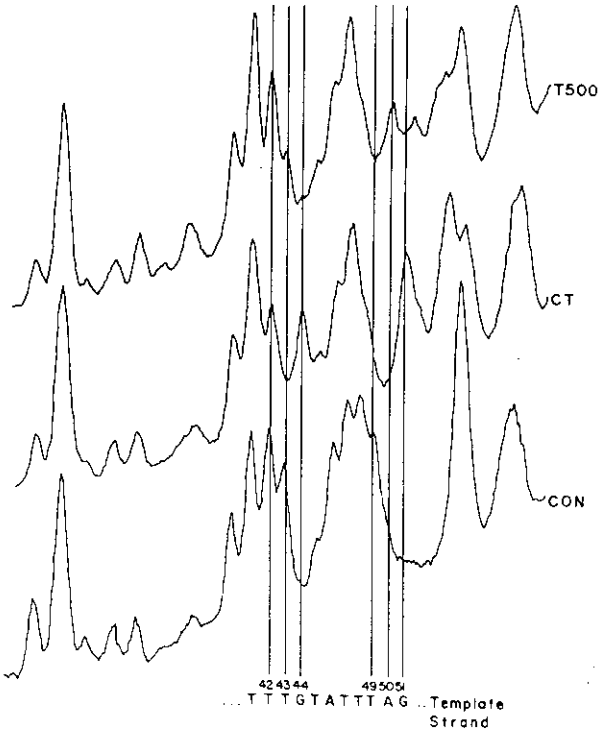


Fig. 7. - Bypass of ultraviolet-induced pyrimidine dimers. Synthesis was accomplished to opposite the 3' base in a dimer by a "second stage" reaction in the presence of Mn^{2+} and dATP (trace 3). This product was taken for reaction with *E. coli* pol I (Kf) and either dCTP plus dTTP (trace 2) or dTTP alone (trace 1). The vertical lines permit visualization of the movement of the bands to opposite the complementary base added, indicating translesion synthesis. (Figure from Rabkin *et al.* [40], reproduced with kind permission).

stabilizes the mismatch. The role of the sequence in determining events at a lesion was dramatically illustrated by Hayes and LeClerc [42] who showed that *in vitro* DNA synthesis terminated opposite the site of thymine glycols except when these glycols were in the sequence 5'-CTPurine-3'. Termination was not observed at such sequences.

It has been suggested by Livneh [43] that polymerase processivity is an important factor in the bypass of lesions. Livneh reports that *E. coli* pol III is able to bypass a fraction of pyrimidine dimers by translesion synthesis as long as it synthesizes in a processive mode. Once the polymerase falls off the substrate, it is unable to reinitiate at the dimer. Our recent studies also indicate the importance of processivity. One of the goals of our work is the development of a system for the *in vitro* study of the specificity of induced mutations. In order to accomplish this goal it is necessary to make sure that the mutational events being studied occur *in vitro* rather than in the host organism in which they are eventually assayed. We are approaching this goal via a modification of the system used by Kunkel [44] for the production of mutation *in vitro* (Fig. 9). A uracil-containing M13 template containing a complementing *lac* insert is prepared by growth of the virus in an *ung⁻ dut⁻* strain. This

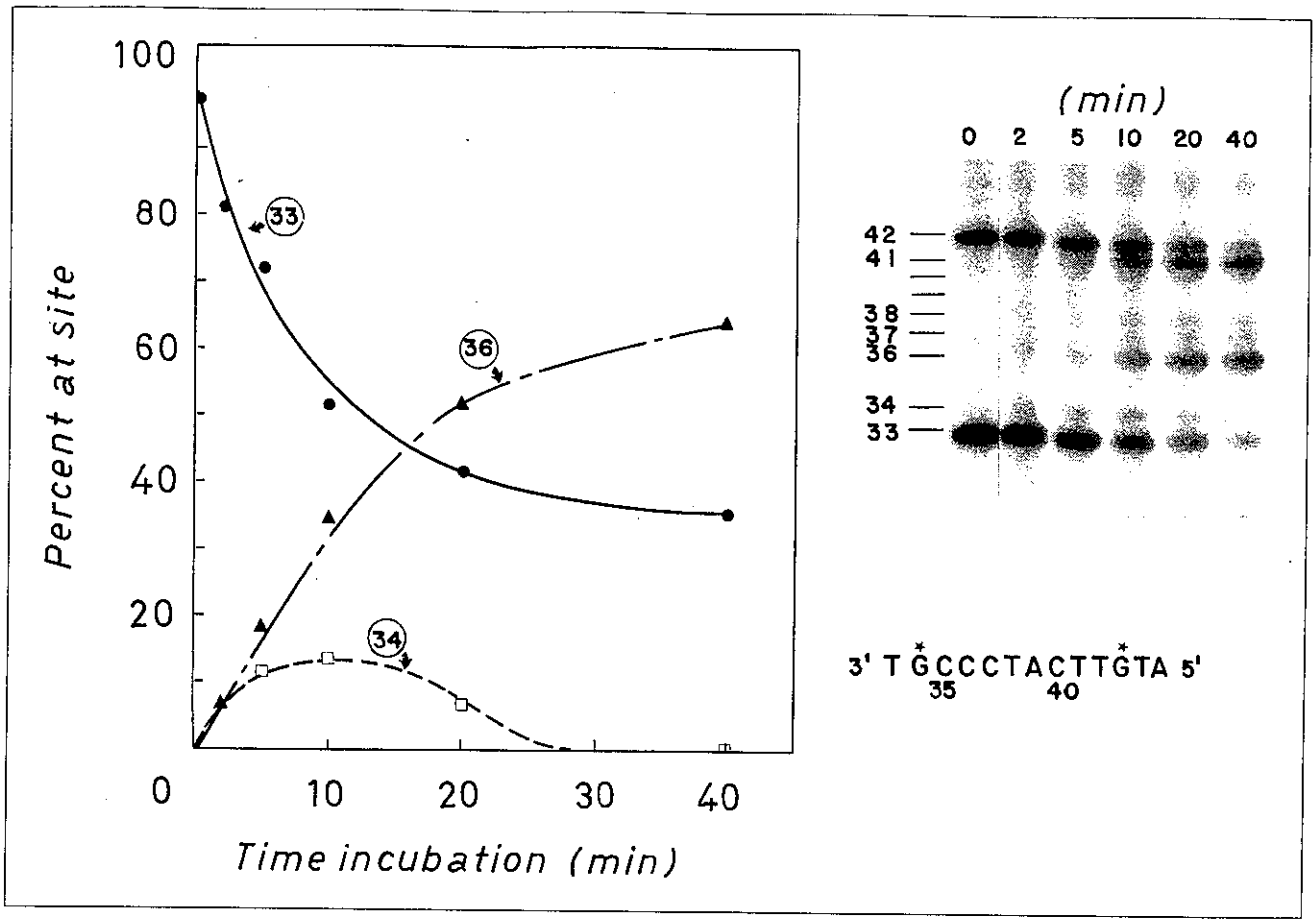


Fig. 8. - Time course of dGTP insertion opposite an AAF-dG adduct. Synthesis was by *E. coli* DNA pol I (Kf) in the presence of Mn^{2+}. The substrate was an acetyl aminofluorene-containing DNA on which synthesis had terminated 3' to the lesion, as shown in the Figure. (Based on data of Rabkin and Strauss [38]).

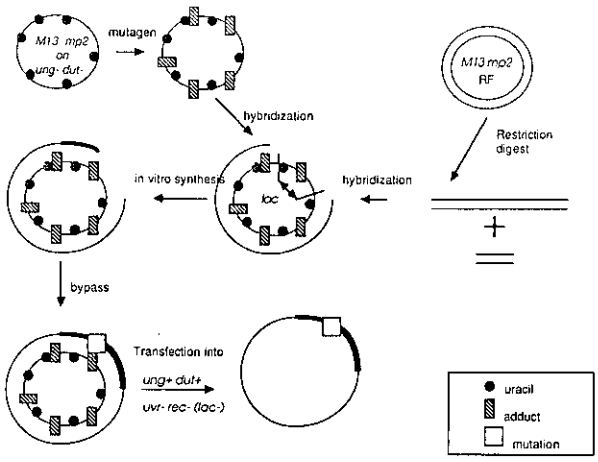


Fig. 9. - Scheme for the detection of mutants produced by *in vitro* synthesis.

molecule is then hybridized with a linear phage DNA fragment from which the *lac* insert has been removed. The resulting gapped molecule transfects poorly since the uracil-containing (+) strand is destroyed on transfection into an *ung*+ *dut*+ recipient. Closing of the gap in the (-) strand by *in vitro* synthesis with normal nucleotides increases transfection efficiency. If mutagenic lesions are present in the uracil-containing gapped region, then only trans-lesion synthesis will produce an efficiently transforming substrate (Fig. 9). We transfect into a *recA* strain to be certain that SOS functions are not induced in the recipient organism.

We used two polymerases with this system. The first, *E. coli* pol I (Kf) has been used in many of our previous experiments. The second polymerase is a T7 DNA polymerase chemically treated to reduce 3'→5' exonuclease activity but containing the thioredoxin protein which makes the T7 enzyme particularly processive (Sequenase) [45]. This preparation retains about one percent of the 3'→5' exonuclease activity of the parent enzyme, which in our hands is about equal to the exonuclease activity of *E. coli* pol I (Kf). Neither of these polymerases appears able to bypass acetyl aminofluorene lesions in DNA *in vitro*. They differ in their ability to produce mutations on a template containing aminofluorene lesions (Table 1). Since we obtained a higher mutation frequency following *in vitro* synthesis with Sequenase as well as lower inhibition of transfection efficiency by aminofluorene lesions (on the template strand) as compared with *E. coli* pol I (Kf) synthesis products, we con-

Table 1. - In vitro *mutagenesis by synthesis on an AF-uracil-containing template*

Sample	Enzyme	pfu counted	pfu/ng DNA	Mutants total	Mutants blue	Mutants % total	Mutants % blue
No synthesis	0	1625	0.16	26	6	1.6	0.37
control	Seq	9057	2.1	87	55	0.96	0.60
AF(2.4)	0	495	0.07	3	1	0.61	0.20
AF(2.4)	Seq	15937	1.9	414	209	2.6	1.3
AF(6)	0	280	0.04	5	3	1.8	1.1
AF(6)	Seq	3312	0.40	178	74	5.4	2.2
No synthesis	0	832	0.18	14	3	1.7	0.36
control	Kf	22827	5.28	64	56	0.28	0.25
AF(2.4)	0	585	0.083	3	0	0.51	0
AF(2.4)	Kf	19642	1.58	89	69	0.45	0.35
AF(6)	0	367	0.052	2	1	0.54	0.27
AF(6)	Kf	1998	0.16	26	8	1.3	0.4

Sequenase and *E. coli* reactions were run at 10 °C. Control templates do not contain AF adducts. Numbers in parenthesis refer to average adducts per molecule calculated from the radioactivity bound to DNA. The sequenase and *E. coli* polymerase experiments were done separately. Seq: modified T7 DNA Polymerase, Sequenase [45]; Kf: *E. coli* DNA polymerase I, Klenow fragment.

clude that Sequenase has greater facility in bypassing aminofluorene lesions *in vitro*. Both enzymes have approximately equivalent exonuclease activities. It therefore seems likely that the key difference lies in the different processivities of the two polymerases. It is interesting that *in vivo*, SOS functions appear to be necessary for the production of mutations in organisms containing aminofluorene lesions although such lesions are themselves poor inducers [46]. Lutgerink *et al.* [47] show that in *E. coli*, the majority of aminofluorene adducts are bypassed even in *umuC* deficient mutants.

The *in vitro* data illustrate how the data of Tessman [33] and of LeClerc *et al.* [34] can be explained and support the hypothesis of Bridges *et al.* [1, 32]. According to this view, SOS proteins need not be critical in a qualitative sense but do serve to facilitate the elongation step of translesion synthesis. Since much of SOS-dependent mutagenesis is targeted, that is at the (putative) site of the lesions in DNA, the specificity of mutagenesis must be determined by the specificity of insertion (or of slippage) opposite lesions. Unless some special and as yet unknown polymerase or modifying proteins can be shown to be activated by SOS induction, the factors determining the specificity of insertion at a particular site can be already be enumerated. They include the nature of the lesion, the innate specificity of the polymerase, the role of the surrounding sequence and the effect of accessory factors including metal ions.

Only certain types of lesion require SOS products and in general these are the lesions which block DNA synthesis. Some years ago we found it convenient to classify changes in base structure as producing instructional or non-instructional sites [48]. An instructional site was defined as containing information which allows for the formation of Watson-Crick base pairs. We supposed that the fact that a lesion blocked DNA synthesis signified that it was non-instructional. Incorporation of base analogs produces an instructional site which results in missense mutations. The production of abasic lesions was taken as a paradigm of a non-instructional site. We demonstrated that dATP is preferentially inserted opposite such abasic lesions, a finding duplicated and amplified in at least two other laboratories. This finding and similar findings led to our statement of what has been called the "A rule", the argument that DNA polymerases when confronted with a non instructional lesion tend to insert adenine nucleotides. This rule derived from *in vitro* studies had been anticipated many years earlier by Tessman [49] as a result of his studies on mutagenic behavior in bacteriophage. The "A rule" applies to many of the mutational specificities worked out in microorganisms but there are exceptions [50]. In addition, even the production of certain frame shifts requires SOS functions *in vivo* [31].

The detailed studies of polymerase insertion opposite abasic sites [51, 52] demonstrate that A insertion is not absolute but rather that there is a bias towards A which varies with the DNA polymerase used (Table 2). It is still not clear whether both bases of the cyclobutane dimer produced by ultraviolet light are non-instructional. Studies with other lesions, of which angelicin is one of the more interesting, indicate that the nature of the lesion and of the sequence context in which the lesion occurs, can determine its instructional nature [50]. It may be that lesions which do provide instructions for base pairing nonetheless result in a structure which cannot be

Table 2. - *Relative efficiency of base insertion opposite an abasic site*

	dNTP				Ref.
DNA Polymerase	A	G	C	T	
α *(Drosophila)*	82.1	11.1	3.2	3.7	52
α (Calf thymus) *(b)*	71.4	14.8	2.6	9.2	51
AMV Rev. transcriptase	88.9	7.0	0.2	3.9	51

(a) Data of Randall *et al.* [52] giving the relative efficiency (Vmax/Km) for the insertion of the four different dNTP's into an abasic site with four different nearest 3' neighbors were averaged. The averaged relative efficiencies were then normalized to 100 percent to give the Figures shown.
(b) The data of Takeshita *et al.* [51] for an abasic site in the tetrahydrofuran configuration have been used.

elongated after the addition of a nucleotide opposite the lesion. An example from our own work is the ability of dC to be added opposite acetylaminofluorene lesions even thought the resulting product is unable to elongate [38]. Our data do not distinguish the occurrence of a rare instructional base pairing, which then permits polymerase action, from the ability of particular polymerases to induce a specific base conformation. One of the unsolved questions of mutagenesis research is whether the polymerase plays an active role in the selection of bases inserted opposite an altered site; *i.e.* whether truly non-instructional sites occur. The demonstration [53] that it is possible to construct base pairing mechanisms in which the *anti* form of a base pairs with the *syn* form does not demonstrate that such base pairing is the basis of the polymerase catalyzed insertion. Indeed the kinetic measurements of Randall *et al.* [52] indicate that at least for insertion at abasic sites, the polymerase itself is involved in the base selection.

Could mutational specificity in SOS mutagenesis be solely the result of the normal specificity rules operating on polymerases in their interaction with particular templates? Our *in vitro* data suggest that this is certainly possible. We sequenced the mutants obtained with sequenase after synthesis past aminofluorene lesions (Table 1) and compared the changes with an equal number of spontaneous mutants occurring as a result of synthesis on an unreacted uracil-containing template. As can be seen, the distribution of mutations observed is quite different in the two cases (Table 3). Twenty percent of the spontaneous mutations are C→T transitions as compared to three percent of the mutants obtained after synthesis past the aminofluorene containing templates. We suspect that the high spontaneous frequency of C→T transitions in the control is the result of spontaneous deamination which goes unrepaired during growth of the virus in an *ung⁻ dut⁻* strain. Twenty six percent of the spontaneous mutants were transitions compared to six percent in the induced sample. Both sets contained equal proportions of transversions but (so far) all of the transversions from the AF-containing template have been G→T. Thirty percent of the spontaneous mutations are deletions as compared to forty eight percent in the induced sample; thirty percent of the spontaneous deletions involve G as compared with eighty seven percent of the induced. The aminofluorene adducts in our substrate are exclusively (> 98 percent) on the C-8 of guanine with 2.2 percent of the adducts in the ring-open-

Table 3. - *Summary of sequencing results*

		Control	AF-containing
Transversions	G→T	21	29
	A→T	4	0
	G→C	3	0
	T→G	1	0
Transitions	G→A	0	2
	A→G	3	0
	C→T	13	2
	T→C	1	0
Deletions (3'→5')	G	4	16
	C	1	0
	A	7	0
	T	3	0
	TG	0	2
	GC	2	7
	GT	0	1
	AC	1	0
	AT	1	1
	CA	1	1
	GCA	0	1
	ACA	0	1
	multiple	0	1
Total number of mutants		59	60
Number of mutations observed		66	64

The Table summarizes the sequencing results to May 1988. The changes were observed in mutants obtained by transfection of uracil-containing M13mp2 gapped templates following synthesis with sequenase (control) or after synthesis with sequenase on a gapped template containing an average of 6 aminofluorene adducts per molecule.

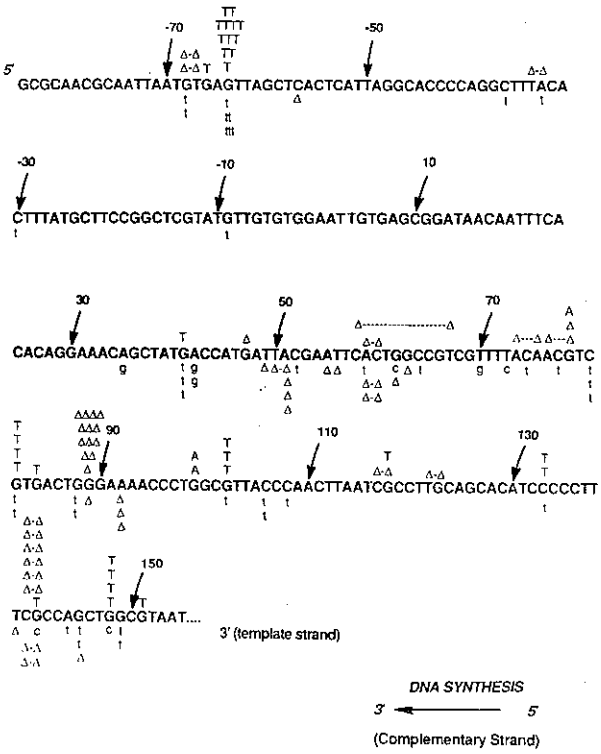


Fig. 10. - Summary of sequence data as of May, 1988. The location and nature of the mutants sequenced is indicated above or below the sequence. Lower case letters or symbols below the sequence indicate mutants obtained after synthesis on an untreated, uracil-containing gapped template. Upper case letters and symbols above the line indicate results with mutants obtained by synthesis on an aminofluorene-uracil-containing, gapped template. The numbers indicated by arrows are the nucleotide positions for the sequence as given by Kunkel [54]. Δ deletion; Δ----Δ deletion spanning a sequence.

ed form (we thank Dr. Moon-shong Tang for this analysis.) The pattern of mutations shows hot spots within the sequence (Fig. 10) [54] but we have not observed any regularities other than that the sequence 5'CCCAAA AGGG3' is a hot spot for -1 G deletions. Even though the frequency of mutants is only five times higher in the AF-containing template than in the control, the pattern of mutation is distinctive. The AF-containing template yields an excess of G→T transversions and of (-1) frameshifts involving mainly G. *In vivo*, mainly G→T transversions produced by aminofluorene lesions and (-1) G frameshift mutations produced by acetyl aminofluorene lesions were found [31, 46]. In *Salmonella typhimurium*, C-8 guanine aminofluorene is an efficient frameshift mutagen [55, 56]. Therefore, a simple system with a polymerase as the only protein can approach the pattern seen *in vivo*. Our experiments make it plausible to suppose that the specificity of SOS repair can be accounted for by the specificity of polymerases as modified by the particular lesion and the surrounding sequence. That is not to say that polymerases can not differ in intrinsic specificity on non-instructional templates so that different mutational patterns might be observed in different organisms. However, it seems possible that the inducible non-polymerase products in *E. coli* are not concerned with the specificity step. According to this view such inducible products are involved in the elongation reactions in *E. coli*. Different organisms may have constitutive factors present which permit elongation. The traditional hypothesis which ascribes error proneness to the inducible factors requires that organisms with a constitutive SOS mutational pathway should have a higher mutation rate. The view that the inducible factors in *E. coli* play a role in elongation (and do not themselves make synthesis error prone) makes this assumption unnecessary.

The detailed biochemical mechanism of inducible mutagenesis in *E. coli* remains to be worked out. The function of the *umuC-D* products, the accessory roles of the recA protein, the question of whether polymerases other than the replicative pol III play a role, the mechanism by which A's are inserted and the role of the following sequence in particular cases all require detailed investigation. Nonetheless, it is now possible to see some of the important consequences of the new information for our knowledge of mutation. First, it is clear that although many organisms possess components of the SOS repair system, those products related to mutagenesis are far more limited in their distribution. The properties of the SOS system as far as mutation is concerned stem from the kinetic requirements for the elongation of a newly synthesized strand past the site of a non-instructive lesion. Rather than the insertion of nucleotides opposite a lesion being the problem, it is the subsequent step which requires additional gene products. The role of exonuclease mutants as mutators is seen as a related but different phenomenon: rapid elongation can substitute for low exonuclease activity in increasing the number of mutations. Finally, the view that it is elongation factors which are induced makes it easier to understand how different species may have constitutive rather than inducible genes to accomplish some of the same purposes. Mutation of such genes would also lead to mutagen stability but without an inducible mutation producing process which is what may be observed in yeast and in mammalian cells.

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