

MUTREV 07318

## Spontaneous mutagenesis: Experimental, genetic and other factors

Kendric C. Smith

*Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305-5105 (USA)*

(Received 8 July 1991)

(Revision received 25 October 1991)

(Accepted 29 November 1991)

**Keywords:** Spontaneous mutagenesis; DNA damage, spontaneous, origins of; Experimental conditions; Origins of spontaneous mutations; Replication errors; Recombination errors; Repair errors; Random mutagenesis; Directed mutagenesis; Spontaneous carcinogenesis

### I. Summary

Spontaneous mutations are “the net result of all that can go wrong with DNA during the life cycle of an organism” (Glickman et al., 1986). Thus, the types and amounts of spontaneous mutations produced are the resultant of all the cellular processes that are mutagenic and those that are antimutagenic. It is not widely appreciated that the types and frequencies of spontaneous mutations change markedly with subtle changes in experimental conditions.

All types of mutations are produced spontaneously, i.e., base substitutions, frameshifts, insertions and deletions. However, very few papers have appeared that are devoted exclusively to the study of the mechanisms of spontaneous mutagenesis, and of the subtle experimental factors that affect the types and frequencies of spontaneous mutations. This is unfortunate because spontaneous mutagenesis appears to play a major role in evolution, aging, and carcinogenesis.

This review emphasizes subtle experimental variables that markedly affect the results of a spontaneous mutation experiment. A thorough understanding of these variables eliminates the need for a theory of “directed” mutagenesis. The intrinsic instability of DNA, and the types of normal metabolic lesions that are produced in DNA that lead to mutations via errors made in replication, repair, and recombination are reviewed, as is the genetic control of spontaneous mutagenesis. As with spontaneous mutagenesis, spontaneous carcinogenesis can also be considered to be the net result of all that can go wrong with DNA during the life of an organism.

---

### II. Introduction

Spontaneous mutagenesis is the most important topic in the science of mutagenesis, since

spontaneous mutations appear to play a major role in spontaneous carcinogenesis (e.g., Totter, 1980; Loeb, 1989; Rudiger, 1990; Ames and Gold, 1991; Loeb, 1991), in aging (e.g., Kirkwood, 1989; Mullaart et al., 1990; Sohal and Allen, 1990; Ames and Gold, 1991), and in evolution (see Section VII). In addition, a thorough understanding of the molecular mechanisms of spontaneous mutagenesis, and of the many factors that affect

---

Correspondence: Dr. Kendric C. Smith, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305-5105 (U.S.A.), Tel. (415) 723-5601; Fax (415) 723-2712.

spontaneous mutagenesis, is required in order to evaluate accurately the responses of cells and organisms to low doses of chemical and physical mutagens, as a basis for regulating the exposure of the public to such agents.

Unfortunately, there aren't many papers in the literature devoted exclusively to spontaneous mutagenesis. Actually, much of the available data on the genetic control of spontaneous mutagenesis has had to be derived from data on untreated cells taken from papers focused on chemical and physical mutagens (e.g., review by Sargentini and Smith, 1985).

The goals of this review are to describe (1) the types of mutations that are produced 'spontaneously', (2) the subtle modifications in experimental procedures that affect markedly the yield and types of spontaneous mutations, (3) the intrinsic instability of DNA and the nature of the DNA damage that produces spontaneous mutations, (4) the roles in spontaneous mutagenesis of errors made by the different mechanisms for processing DNA (i.e., replication, repair and recombination), and (5) to discuss briefly the role of spontaneous mutagenesis in spontaneous carcinogenesis.

It will be clear from the data presented herein that the spontaneous mutation rate of an organism is the resultant of all exogenous and endogenous factors that are either mutagenic or antimutagenic, and that this balance between mutagenesis and antimutagenesis is extremely dependent upon the conditions of the experiment used to measure the spontaneous mutation frequency, and is not the same for all genes or for different types of mutations in the same gene.

Other reviews and discussions on spontaneous mutagenesis have appeared (e.g., von Borstel, 1969; Kondo et al., 1970; Kondo, 1973; Cox, 1976; Quah et al., 1980; Loeb and Kunkel, 1982; Lawrence, 1982; Drake et al., 1983; Sargentini and Smith, 1985; Loeb and Preston, 1986; Ramel, 1989; Meuth, 1990; Drake, 1991a; Sankaranarayanan, 1991). [NOTE: The numerous papers on shuttle vectors are not reviewed here, largely because of the high spontaneous mutation rates for these vectors just from entering and leaving cells, and because of the necessity of limiting the scope of this review.]

### III. Types of mutations that occur spontaneously

Hartman et al. (1971) classified 83 spontaneous histidine auxotrophs of the bacterium *Salmonella typhimurium* and found that 53% were base-substitution mutations, 11% were frame-shifts (i.e., deletions or insertions of one or a few base pairs), 23% were caused by deletions (i.e., deletions of more than a few base pairs), and 13% were apparently (as discussed in Sargentini and Smith, 1985) caused by insertions of large DNA elements. The listed proportion of base-substitution mutations is probably an underestimate, since these studies would have detected only the mutations that inactivate a gene product. One can presume that many missense mutations were not measured in these studies, since they would have had little or no effect on the measured phenotype, i.e., they were silent mutations. The conclusion that base substitutions are the most common form of spontaneous mutation in bacteria, is also valid for yeast (reviewed in Sargentini and Smith, 1985).

There is now a new, but very limited, body of data on the frequencies of the different types of spontaneous mutations, based on DNA-sequencing studies, e.g., yeast (Giroux et al., 1988; Lee et al., 1988), *S. typhimurium* (O'Hara and Marnett, 1991), *Escherichia coli* (Albertini et al., 1982; Fix and Glickman, 1987; Fix et al., 1987; Schaaper et al., 1986, 1987; Gordon et al., 1991), and Chinese hamster ovary cells (de Jong et al., 1988; Phear et al., 1987).

Based upon all of these data, it is clear that all types of mutations can be formed 'spontaneously', but numerous factors, both experimental and genetic (see below), can affect the frequencies and the different types of spontaneous mutations observed in a given study. Therefore, before one postulates some new mechanism of spontaneous mutagenesis such as self-directed mutagenesis (see Section VII), one should first be very sure that the "unusual" results obtained are not just the result of an unusual experimental protocol.

### IV. Experimental conditions that affect mutagenesis

Many simple and often overlooked parameters in experimental protocols can alter markedly the

results of a spontaneous mutagenesis experiment. The type of growth medium (or lack of same), the type of agar, the ambient laboratory lighting, the mutation assay (i.e., the gene under study and the type of mutation being scored), and the genetic background of the cells all have profound effects on the results of a spontaneous mutation experiment.

(1) *Growth medium and growth rate*

(a) Repair-proficient *E. coli* show about a 10-fold greater spontaneous mutation frequency [equivalent to that for excision-repair deficient (*uvr*) strains] when grown in rich medium (e.g., yeast extract-nutrient broth) versus minimal medium. The *uvr* strains do not show this medium effect. This result explains why early workers who used rich growth media found no enhancement of spontaneous mutagenesis in *uvr* strains (reviewed in Sargentini and Smith, 1981).

Since this medium effect is not observed in *uvr* cells, it suggests that the rich medium may be inhibiting an error-free pathway of excision repair in the wild-type strain, or that it enhances metabolic damage to DNA. However, the more probable explanation is that the extra amount of sister duplexes in the more rapidly growing cells in rich medium may lead to more excision-repair events that utilize recombination between sister duplexes (i.e., *recA*-dependent excision repair that is independent of replication; see Smith and Sharma, 1987), and thus increase the spontaneous mutation frequency. Such *recA*-dependent excision repair has been shown to be mutagenic after UV irradiation (Nishioka and Doudney, 1970; Bridges and Mottershead, 1971).

In addition, rich growth medium inhibits an apparently error-free pathway of postreplication repair after the UV irradiation of *E. coli uvrA* or *uvrB* cells, regardless of the type of medium they were grown in prior to UV irradiation. The rich medium can be just minimal medium plus Casamino Acids (Difco) at 2 mg/ml or the 13 pure amino acids therein (Barfknecht and Smith, 1977; Sharma et al., 1982; Sharma and Smith, 1985).

(b) In rich growth medium, the *mutD* strain of *E. coli* (deficient in the proofreading subunit of

Pol III; see Section VI.1.d) shows a several thousand-fold increase in spontaneous mutation frequency over wild-type cells, compared to only a 10–50-fold increase when grown in minimal medium (Erlich and Cox, 1980; and refs. therein). Schaaper (1988) has also observed a striking difference in the spontaneous mutation frequencies of a *mutD5* strain when grown in rich versus minimal medium (Table 1). Furthermore, he supplies evidence that rich medium interferes somehow with *mutHLS* mismatch repair in the *mutD* strain (Schaaper, 1988, 1989).

(c) In glucose-limited cultures of *E. coli* WP2, the spontaneous mutation frequency (Trp reversion) was directly proportional to the growth rate (i.e., from 0.1 to 0.8 generations/h there was a ~6-fold increase in spontaneous mutation frequency; see possible explanation above) (Savva, 1982). Also, the type and concentration of sugar used in the growth medium can affect the spontaneous mutation frequency (Lee and Cerami, 1990).

(d) Nondividing (nutrition limited) cultures of *E. coli ada ogt* cells (deficient in two methyltransferases) showed a 3-fold increase in spontaneous mutagenesis compared with wild-type cells, but their spontaneous mutation frequency did not differ from that for wild-type cells during normal growth phase (Rebeck and Samson, 1991). Therefore, different types (amounts?) of mutagenic damage to DNA (e.g., alkylation) occur under nongrowth conditions. Furthermore, the synthesis of many new types of proteins occurs in starved bacteria (Groat et al., 1986). Clearly, starvation conditions can alter the results of a spontaneous mutagenesis experiment (see Section VII).

(e) For spontaneous mutagenesis in *E. coli*, there was a maximum yield at a temperature of 42°C (from 20 to 42°C there was a ~6-fold increase in spontaneous mutation frequency;  $Q_{10} = 2$ ), and a maximum yield at a pH of 6.0 (from pH 6 to pH 7–8 there was about a 2-fold decrease in spontaneous mutation frequency) (Savva, 1982).

TABLE 1  
GENETIC CONTROL OF SPONTANEOUS MUTAGENESIS IN BACTERIA<sup>a</sup>

Mutation or treatment	Defect(s)	Effect on spontaneous mutagenesis	Mutation assay	Reference
<i>Increases (mutator)</i>				
<i>ada ogt</i>	methyl transferases	1-fold (growth) 3-fold (no growth)	<i>his</i> <sup>-</sup> → His <sup>+</sup>	Rebeck and Samson, 1991
<i>dam-4</i>	DNA adenine methylase, mismatch repair	20-fold	<i>lacI</i> → Lac <sup>+</sup>	Glickman, 1979
<i>dnaE(Ts)</i> <i>mutD(dnaQ)</i>	DNA polymerase III ε-subunit of Pol III	3–15-fold 12-fold (MM) <sup>b</sup> 4400-fold (RM) <sup>c</sup> 130-fold (MM) 3000-fold (RM)	<i>azaU</i> <sup>S</sup> → <i>azaU</i> <sup>r</sup> Nal <sup>S</sup> → Nal <sup>r</sup>	Hall and Brammar, 1973 Schaaper, 1988
<i>mutH,L,S</i>	methyl-directed mismatch repair	260-fold	<i>lacI</i>	Schaaper and Dunn, 1991
<i>mutM</i>	8-oxoguanine DNA glycosylase	14-fold	<i>lacZ</i> → Lac <sup>+</sup>	Cabrera et al., 1988
<i>mutT</i>	dGTP triphosphatase	~ 160-fold ~ 1090-fold	Nal <sup>S</sup> → Nal <sup>r</sup> Str <sup>S</sup> → Str <sup>r</sup>	Bhatnagar and Bessman, 1988
<i>mutY</i>	adenine glycosylase (Au et al., 1989)	~ 175-fold ~ 23-fold	Lac <sup>-</sup> → Lac <sup>+</sup> Rif <sup>S</sup> → Rif <sup>r</sup>	Nghiem et al., 1988
<i>ΔoxyR</i>	regulon for oxidative stress defenses	10–55-fold <sup>d</sup>	<i>his</i> <sup>-</sup> → His <sup>+</sup>	Storz et al., 1987
<i>polA1</i>	DNA polymerase I	100-fold	His <sup>+</sup> → <i>his</i> <sup>-</sup>	Jankovic et al., 1990
<i>sodA sodB</i>	superoxide dismutases	5-fold	Thy <sup>+</sup> → Thy <sup>-</sup>	Farr et al., 1986
<i>ssb-113</i>	single-strand binding protein	10-fold	<i>trp</i> → Trp <sup>+</sup> <i>argE</i> → Arg <sup>+</sup>	reviewed in Meyer and Laine, 1990
<i>uvrB5</i>	nucleotide excision repair	2-fold 6-fold	<i>trpE9777(fs)</i> → Trp <sup>+</sup> <i>lacZ53(UAG)</i> → Lac <sup>+</sup>	Sargentini and Smith, 1981
<i>ΔuvrD</i>	helicase II	100-fold	Spc <sup>S</sup> → Spc <sup>r</sup>	Washburn and Kushner, 1991
<i>xth nth nfo</i>	base excision repair, AP sites	5-fold	<i>argE3</i> → Arg <sup>+</sup>	Cunningham et al., 1986
<i>Decreases (antimutator)</i>				
anoxia	oxidative metabolism	~ 2-fold	several assays	R.G. Fowler, 1991, personal communication
auxotrophy	Pur <sup>-</sup>	0.3-fold <sup>d</sup>	<i>his</i> <sup>-</sup> → His <sup>+</sup>	Storz et al., 1987
	Thr <sup>-</sup>	13-fold	Val <sup>r</sup>	Quinones and Piechocki, 1985
	Ser <sup>-</sup>	3-fold		
<i>lexA101</i>	repressor of SOS functions	14-fold 2-fold	<i>lacZ53(UAG)</i> → Lac <sup>+</sup>	Sargentini and Smith, 1981
<i>umuC36</i>	UV radiation mutagenesis	11-fold <sup>c</sup>	<i>lacZ53(UAG)</i> → Lac <sup>+</sup>	Sargentini and Smith, 1981
<i>uvrD3</i>	helicase II	6-fold <sup>c</sup>	<i>lacZ53(UAG)</i> → Lac <sup>+</sup>	Sargentini and Smith, 1981
<i>recA56</i>	recombination, SOS response	2-fold	<i>lacZ53(UAG)</i> → Lac <sup>+</sup>	Sargentini and Smith, 1981
		16-fold <sup>c</sup> 25-fold	<i>lac</i> <sup>-</sup> → Lac <sup>+</sup> (long deletion)	Albertini et al., 1982

(f) Ames and co-workers (Ames, 1983; Ames et al., 1990) have listed numerous mutagens and antimutagens in a wide variety of foodstuffs, and some of these mutagens and antimutagens may be present in certain types of culture media derived from such foodstuffs (e.g., the various types of broth and cell extracts used to culture bacteria). In fact, Maron and Ames (1983) have abandoned the use of nutrient broth (Difco) with *Salmonella* tester strains TA1535 and TA100, because they observed mutations in these strains in the absence of S9. They switched to Oxoid nutrient broth No. 2. These authors conclude that "Unfortunately, any nutrient broth that contains proteins extracted from beef at high temperatures is likely to be mutagenic to some extent".

(g) Of the 19 amino acids tested (at 2 mM) for mutagenicity in *E. coli* K12 *uvrB* [*lacZ53*(UAG) → Lac<sup>+</sup>], cystine and, to a lesser extent, arginine and threonine were found to be antimutagenic, while only phenylalanine was found to be mutagenic. Tyrosine and, to a lesser extent, tryptophan (each at 2 mM) inhibited the mutagenicity of phenylalanine. Phenylalanine was not mutagenic in a wild-type strain, suggesting that the DNA lesions produced by the metabolism of phenylalanine are repaired by the error-free mode of nucleotide excision repair (Sargentini and Smith, 1986). Other studies have also demonstrated that the metabolism of amino acids can be mutagenic (e.g., Quinones and Piechocki, 1985; Glatt, 1990).

(h) *E. coli* grown to log phase in minimal medium are much less able to repair DNA double-strand breaks after X-irradiation than are cells grown to log phase in rich growth medium ("medium-dependent resistance"; Sargentini et al., 1983). In addition, a *radA100* mutation sensitizes *E. coli* to killing by X- and UV-irradiation when

grown in rich growth medium, but not if grown in minimal medium (Diver et al., 1982). This suggests that the *radA100* gene plays an important role in "medium-dependent resistance". Similarly, cells grown to stationary phase in nutrient broth plus glucose are much more resistant to ionizing radiation than are such cells grown without added glucose ("glucose-induced resistance"; Friesen et al., 1970). These are further examples of how subtle changes in growth conditions can have a profound effect on the ability of a cell to repair DNA damage.

### (2) Agar

Impurities in most brands of agar have a selective inhibitory effect on the *recA*-dependent pathway of excision repair (van der Schueren et al., 1974). In this case, since the impurities in the agar are inhibiting the mutagenic pathway of excision repair, i.e., the *recA* branch (Nishioka and Doudney, 1970; Bridges and Mottershead, 1971), such impure agars should *reduce* spontaneous and other types of mutagenesis, but this has not been tested directly. To avoid such problems, our studies on spontaneous mutagenesis used high purity Noble agar (Difco), whose lack of effect on *recA*-dependent excision repair was tested before use (Sargentini and Smith, 1981).

### (3) Lighting

Ambient laboratory lighting conditions should not be ignored. Nutrient agar medium is made highly toxic to certain repair deficient strains of *E. coli* (e.g., *recA*, but not *uvr* or wild-type) by exposure to cool white or to near-UV (black light) fluorescent lamps (Webb and Lorenz, 1972). Furthermore, visible light ( $\lambda < 500$  nm) is mutagenic for continuous cultures of *E. coli* (Webb and Malina, 1970), and for cells growing on plates (McGinty and Fowler, 1982). Cool white fluores-

---

#### Notes to Table 1:

<sup>a</sup> Data are for *E. coli* unless otherwise specified. This table does not include all available data on the mutations that affect DNA repair and other metabolic functions that also affect spontaneous mutagenesis. Such a list is available through 1984 (Sargentini and Smith, 1985). This table includes new data, and repeats some of the earlier data where needed for supporting the text.

<sup>b</sup> MM, minimal growth medium.

<sup>c</sup> RM, rich growth medium.

<sup>d</sup> *S. typhimurium*; range depends upon the strain and the presence of plasmid pKM101.

<sup>e</sup> *uvrB5* background.

cent lights are also mutagenic for *Salmonella* (Hartman et al., 1991). As a precaution, we protect all rich media, agar plates, and stock amino acids by covering bottles with aluminum foil, or by storing them in the dark, or by using gold fluorescent lamps ( $\lambda > 500$  nm) in storage areas (e.g., cold room), and in the laboratory during the handling of cells.

#### (4) Mutation assay

(a) In addition to the many experimental factors listed above that affect the yield of spontaneous mutations, no factor is more important than the mutation assay itself, i.e., what gene is being studied and what types of mutations are being screened (e.g., DeMarini et al., 1989). Just to cite one example where the differences were small *but real*, spontaneous mutagenesis in *E. coli* K12 *uvrB5*, relative to its isogenic wild-type strain varied from a 1.9-fold increase [*trp-E9777(fs)*  $\rightarrow$  Trp<sup>+</sup>] to a 6.2-fold increase [*lacZ53(UAG)*  $\rightarrow$  Lac<sup>+</sup>] (Sargentini and Smith, 1981). Many examples of these types of differences in results depending on the assay used are available from the literature on chemical and radiation mutagenesis (e.g., Sargentini and Smith, 1989).

(b) Of no less importance than the inherent differences in the mutation frequencies of different genes, is the large difference in spontaneous mutation frequency in a given gene depending upon whether it is functioning in transcription or not. Thus, genes show a 15–150-fold higher frequency of spontaneous mutagenesis when derepressed (Korogodin et al., 1991; see also Davis, 1989). This is apparently due both to the fact that DNA turnover occurs at sites of transcription (Grivell et al., 1975), and that the nontranscribed strand of an active gene is considered to be single-stranded, and single-stranded DNA is much more susceptible to chemical alteration (e.g., deamination, depurination) than is double-stranded DNA (see below).

(c) Another factor that potentially can have a profound effect on spontaneous mutagenesis is whether the spontaneous lesion appears in the

gene of interest before or after the gene is replicated. There are many more opportunities for mutagenic DNA repair and replication for a damaged gene that is about to be replicated than there is for a gene that has already been replicated (e.g., Smith and Sharma, 1987).

(d) It is also important to point out that the base sequence around a given base pair can also affect the frequency of mutation events at a particular base pair, i.e., the “context” or “next-nucleotide” effect (e.g., Fix et al., 1987; Drobetsky et al., 1987; Phear et al., 1987; Goodman, 1988).

#### (5) Genetic background

The genetic background of a cell can have a profound effect on the amount of spontaneous mutations observed under a given set of experimental conditions. Table 1 lists some mutations in *E. coli* and *S. typhimurium* that affect survival under various types of stress, and their additional effects upon spontaneous mutagenesis (both increases and decreases) versus a wild-type strain.

If the genetic backgrounds of cells being compared are not strictly isogenic, which is often quite difficult or impossible to achieve during strain construction, subtle deviations from isogenicity could have a profound effect on the results. On the other hand, known differences in genetic background can be used knowledgeably to give important information on the sources of spontaneous mutations.

While the data in Table 1 provide a beginning toward understanding which DNA repair and metabolic events lead to spontaneous mutagenesis, it is not possible to quantitatively rank these several processes to determine their relative importance in spontaneous mutagenesis, because generally the experiments were performed upon different strains of *E. coli* grown under different conditions, and different assays for spontaneous mutagenesis were used. What would be ideal would be to repeat these experiments in a set of truly isogenic strains, except for the mutation under study, and to use the same experimental conditions, and the same mutation assay.

However, one problem with this approach is the fact that even though the spontaneous muta-

tion frequency increases markedly in a strain deficient in a specific repair function, this does not prove that this specific DNA lesion is important to spontaneous mutagenesis in the wild-type strain. For example, if the wild-type strain is fully able to repair this type of lesion accurately, then it would not contribute to the spontaneous mutation frequency of the wild-type strain, but would only become important in the repair-deficient strain.

One way of testing the competence of the wild-type strain to repair a specific type of spontaneous lesion is to overproduce a particular repair enzyme in the wild-type strain, and then to see if this further decreases the spontaneous mutation frequency of the wild-type strain (see Section V.6). Obviously, in this type of experiment, the numbers of mutants may keep getting smaller, and thus become more difficult to determine with accuracy. In addition, the overproduction of some gene products can be toxic.

Although most of the available data on the genetic control of spontaneous mutagenesis are concerned with mutator mutations, i.e., mutations that enhance spontaneous mutagenesis, an equally valid approach is to study *antimutator mutations*, i.e., mutations that inhibit spontaneous mutagenesis (e.g., Geiger and Speyer, 1977; Quah et al., 1980; Quinones and Piechocki, 1985). Consistent with the model that much of spontaneous mutagenesis is due to damage to DNA by normal metabolism (e.g., Sargentini and Smith, 1981), several antimutator mutations are those that block the synthesis of purines, threonine and serine (Table 1).

#### (6) *Insertion elements*

In the late 1940's, Barbara McClintock (Nobel Prize in 1983) discovered mobile genetic elements. These elements regularly jump to new locations in a chromosome, and often inactivate the genes into which they become inserted. Insertion sequences in procaryotes are simple transposons, i.e., transposable elements that carry no genetic information except that necessary for transposition. A complex transposon carries additional genetic material unconnected with transposition, e.g., antibiotic resistance genes. Fre-

quently, DNA-sequencing data for mutagenesis experiments will report a percentage of the total mutants as being due to insertion elements (e.g., Schaaper et al., 1986). The importance of insertion elements in spontaneous mutagenesis depends upon whether the cell culture under investigation contains such elements, and whether at the time of the experiment these elements happen to reside in the gene under investigation.

## V. Origins of spontaneous DNA damage

### (1) *Metabolism of amino acids and sugars*

One does not need to evoke the topic of man-made chemicals and pesticides to have plenty of candidates for the causes of spontaneous mutagenesis and spontaneous carcinogenesis. For example, plants have their own pesticides as a natural defense against predators, and plants are a major source of our food (e.g., Ames, 1983; Ames et al., 1990). However, natural pesticides in food are not the only source of mutagens, even the metabolism of a required amino acid (e.g., phenylalanine; Sargentini and Smith, 1986) can be mutagenic (see also, Quinones and Piechocki, 1985; Glatt, 1990), the metabolism of sugars can be mutagenic (Lee and Cerami, 1990), and the metabolism of oxygen can be mutagenic (see below). The concept of normal metabolism as a major source of spontaneous mutagenesis (e.g., Sargentini and Smith, 1981) is now also being considered as the major source of spontaneous carcinogenesis (e.g., Loeb, 1989; Rudiger, 1990).

### (2) *Deamination*

In addition to DNA damage caused by the metabolic production of mutagenic byproducts of normal metabolism, one must also be concerned with the intrinsic instability of DNA as one cause of spontaneous mutagenesis. For example, the spontaneous deamination of cytosine residues occurs at the rate of 15/h per mammalian cell for double-stranded DNA and 4000/h for single-stranded DNA (reviewed in Billen, 1990). The mutagenic consequences of spontaneous deamination will be discussed below in Section VI.1.b (Miscoding lesions).

### (3) Depurination and depyrimidination

Depurination occurs at the rate of 1000/h per mammalian cell in double-stranded DNA and 4000/h in single-stranded DNA. The rate for depyrimidination is 50/h for double-stranded DNA and 200/h for single-stranded DNA (reviewed in Billen, 1990). Since apurinic and apyrimidinic sites (AP sites) are mutagenic (e.g., Schaaper and Loeb, 1981; Loeb and Preston, 1986), one would predict that there should be an enhanced spontaneous mutation frequency due to AP sites in the single-stranded, nontranscribed strand of an active gene, but such mutagenesis data are not known to this author. The role of AP sites in spontaneous mutagenesis will be discussed below in Section VI.1.c (Noncoding lesions).

### (4) Endogenous methylation

S-Adenosylmethionine is a source of methyl groups for a number of biosynthetic pathways in cells, e.g., epinephrine,  $\gamma$ -methylglutamate, methyl-accepting chemotaxis proteins, and the methylation of cytosine and adenine in DNA. It is not known how frequently such methylations occur on the wrong molecules in cells, but S-adenosylmethionine can act as a weak alkylating agent *in vitro* (Naslund et al., 1983).

Cells are well supplied with enzymes to handle alkylation damage. When *E. coli* are grown in the presence of low levels of alkylating agents, they induce a set of enzymes that renders them resistant to both the mutagenic and killing effects of an acute exposure to the same or another alkylating agent (reviewed in Friedberg, 1985).

In an *ada ogt* mutant of *E. coli*, which is deficient in both the constitutive (*ogt*) and inducible (*ada*) methyltransferases, the spontaneous mutation frequency (His<sup>+</sup> reversion) of the double mutant was the same as that for the wild-type strain during normal growth phase (Rebeck and Samson, 1991). This agrees with the results of Jeggo (1979) for an *ada* strain. However, metabolic methylation mutagenesis increased about 3-fold in the double mutant under *nongrowth* conditions (Rebeck and Samson, 1991). These results emphasize that new types (amounts?) of mutagenic damage to DNA occur

when cells are held under nongrowth conditions (see Section VII).

### (5) DNA turnover

It is quite incorrect to consider that once a cell culture is switched to buffer (or to other nongrowth conditions) that cellular metabolism ceases. Quite the contrary, a considerable amount of metabolism continues. For example, DNA turnover (i.e., continuous DNA degradation and resynthesis without a net change in cellular DNA content) occurs when *E. coli* B/r is held in buffer. Ribosomal RNA degradation may contribute a sizeable fraction of the precursors for this DNA turnover synthesis (Tang et al., 1979).

DNA turnover was reduced 5-fold when *dnaB* cells (temperature-sensitive for DNA replication) were incubated at the nonpermissive temperature, and the residual DNA turnover was inhibited by rifampicin. These results indicate that DNA turnover occurs both at the DNA replication fork and at the sites of transcription (Grivell et al., 1975).

DNA turnover, which can be considered as a type of repair process with the inherent errors of all such processes, may help explain the appearance *with time* of spontaneous mutations under nongrowth conditions (e.g., Hall, 1990).

### (6) Oxygen metabolism: radicals and excited states

Although there are large numbers of books, journals, and meetings devoted to "oxidative damage and repair", there have been few attempts in the literature to *quantitate* the biological importance of oxygen toxicity, e.g., its role in spontaneous mutagenesis.

One project focused on this goal measured the effect of increased oxygen concentrations on an Ames tester strain *S. typhimurium* TA100 ( $\Delta$ *uvrB* pKM101) for *hisG46* reversion (Kelley and Baden, 1980). These authors found a small oxygen dependent increase in mutagenesis that reached a maximum (~35% increase) at about 80% oxygen (air is about 20% oxygen). In another study, the aeration rate of the culture increased the spontaneous mutation frequency about 2-fold (from 0.1 to 1.0 liters of air per minute; Savva, 1982).

In the reverse experiment, where *E. coli* K-12 cells were grown overnight under anaerobic con-



ditions and then plated for several forward and reversion assays, there was about a 2-fold reduction in the spontaneous mutation frequency (R.G. Fowler, 1991; personal communication). When *S. typhimurium* ( $\Delta gal-uvrB$ ) were grown under anoxic conditions (0.1% oxygen or less), 30% fewer small deletions (3- and 6-base pair), and a 5-fold decrease in minus frameshift mutations were observed. In contrast, the frequency of G:C  $\rightarrow$  A:T transition mutations increased about 5-fold (Hartman et al., 1984). Although these authors tested many possible experimental variables that might explain this unusual enhancement of transition mutations, they found no easy explanation. [N.B. Some proteins disappear and new proteins are synthesized when cells are switched to anaerobic conditions (e.g., Smith and Neidhardt, 1983a, b; Hartman et al., 1984; Choe and Reznikoff, 1991).]

The *xth nth nfo* mutant of *E. coli*, which is grossly deficient in AP endonuclease activity and is partially deficient in glycosylase activity, activities that are needed for the repair of several different types of oxidative DNA damage, showed a 5-fold increase over the wild-type strain in spontaneous *argE3* reversion (Cunningham et al., 1986).

Thus, the effect of going from 20% oxygen to  $\sim$  0% oxygen reduced spontaneous mutagenesis about 2-fold. Eliminating a set of major enzymes for the repair of oxygen-dependent DNA damage (i.e., *xth nth nfo*) increased spontaneous mutagenesis in air about 5-fold. However, in going from 20% to 80% oxygen, only about a 35% increase in mutagenesis was observed.

When cells grow aerobically, molecular oxygen can be reduced to yield the superoxide radical, which is converted to hydrogen peroxide by the enzyme superoxide dismutase. An *E. coli* double mutant (*sodA sodB*), completely lacking in superoxide dismutase activity, had a 5-fold greater spontaneous mutation frequency than did the wild-type strain during aerobic growth. This enhanced mutagenesis is *recA*-independent, but is largely dependent upon the presence of functional exonuclease III (*xth*). This suggests that oxidative damage in DNA is enhanced in the absence of superoxide dismutase, and that exonu-

lease III converts this damage to mutagenic lesions (Farr et al., 1986).

Similarly, a null mutant of *Saccharomyces cerevisiae* Cu, Zn superoxide dismutase showed a 4-fold higher spontaneous mutation rate versus wild-type under aerobic growth (TRP<sup>-</sup>  $\rightarrow$  TRP<sup>+</sup>), but "was similar to that of the wild-type" under anaerobic growth (Gralla and Valentine, 1991).

The *oxyR* gene positively controls a regulon involved in cellular defenses against oxidative stress in *S. typhimurium* and *E. coli*. A dominant mutant, *oxyR1*, is resistant to H<sub>2</sub>O<sub>2</sub> and constitutively overexpresses nine H<sub>2</sub>O<sub>2</sub>-inducible proteins. The spontaneous mutation frequency for a strain deleted in *oxyR* was 10–55-fold higher than for the isogenic *oxyR*<sup>+</sup> strain, while the *oxyR1* mutation (overproducing strain) reduced spontaneous mutagenesis by a factor of 2. However, when the *oxyR* deletion strain was grown anaerobically, there was only a 70% reduction in spontaneous mutagenesis, and only a 32% reduction for the wild-type strain, and no effect for the *oxyR1* (overproducing) strain (Storz et al., 1987).

Both experimental approaches seem valid, but the results are not consistent. The 10–55-fold increase (depending on strain and the presence of the mutation-enhancing plasmid pKM101) in spontaneous mutagenesis in the *oxyR* deletion strain compared to the wild-type strain suggests a large role for oxygen in spontaneous mutagenesis, but a 32% reduction in spontaneous mutagenesis in the wild-type strain under anoxia is not an impressive argument that oxygen plays a major role in spontaneous mutagenesis.

One can think of a number of possibilities to explain these data: (1) strictly anoxic growth was not achieved in these experiments, thereby underestimating the role of oxygen, (2) switching cells to anoxic growth initiates a new set of metabolic events (Smith and Neidhardt, 1983a, b; Hartman et al., 1984; Choe and Reznikoff, 1991) that may be as mutagenic as aerobic events, (3) elements of the OxyR regulon may also function to repair DNA lesions that are *not oxygen-dependent*, and (4) the simplest explanation is that oxygen metabolism produces potentially mutagenic damage that is efficiently repaired in the wild-type strain. In fact, the 32% reduction in

spontaneous mutagenesis under anoxia suggests that 68% of spontaneous mutagenesis is *independent* of oxygen metabolism in the wild-type strain.

Although oxygen metabolism is clearly mutagenic, a precise measure of its importance relative to all other mechanisms for producing spontaneous mutagenesis is still lacking. Also, lacking is a clear picture as to the relative importance of oxygen metabolism in producing spontaneous mutations via radical mechanisms or through the production of excited state molecules (see below).

*Radicals.* It is not the purpose of this article to review the voluminous literature concerned with the many chemical and biological studies on the detrimental effects of the free radicals of oxygen, since this has been done (e.g., Simic et al., 1989; Breimer, 1990; and refs. therein). In most cases, reviews on this topic have relied heavily on the literature concerning the chemical and biological effects of ionizing radiation, since, as discussed above, there have been very few studies on mutagenesis by oxygen metabolism per se. However, the urinary biomarkers of thymine glycol, 5-hydroxymethyl uracil and 8-oxoguanine (reviewed in Simic et al., 1989) are good evidence for the production of DNA damage via the metabolic production of hydroxyl radicals. Nevertheless, if these lesions are repaired accurately, no mutations would occur.

*Excited states.* Cells can produce excited state molecules by enzymatic processes that require oxygen, and this offers the possibility for "photochemistry in the dark". Metabolizing cells exhibit low levels of biological chemiluminescence (e.g., Cadenas, 1984; Quickenden et al., 1985), which is diagnostic for the presence of excited-state molecules. Excited-state molecules have also been observed as a consequence of lipid peroxidation in microsomes (Cadenas et al., 1984).

In addition, model systems exist for the enzymatic production of excited-state molecules (reviewed in Cilento and Adam, 1988). For example, using horseradish peroxidase and several different substrates, excited-state ketones are formed in an enzymatic reaction that requires oxygen, and these excited-state ketones can damage DNA (Cilento, 1980) and proteins (Rivas et al., 1984).

[NOTE: The enzyme reaction produces a dioxetane, a cyclic organic peroxide, which decomposes spontaneously to yield an excited-state (triplet) carbonyl compound, and a ground-state fragment. The triplet-state carbonyl can transfer its energy to other molecules, e.g., thymine.]

Based on a study of the genetic control of spontaneous mutagenesis, it was concluded that much of spontaneous mutagenesis (base substitutions) is due to 'UV-like' DNA damage rather than to 'X-ray-like' DNA damage (Sargentini and Smith, 1981, 1985; Smith and Sargentini, 1985), e.g., mutations that block excision repair (primarily error-free) and enhance UV-radiation mutagenesis also enhance spontaneous mutagenesis, but have little or no effect on X-ray-mutagenesis. [NOTE: In general, UV irradiation produces few, if any, DNA-strand breaks by the direct action of the radiation, and the majority of the base damage produced is of the type that causes a distortion in the DNA helix (e.g., pyrimidine dimers). Repair enzymes such as the UvrABC exonuclease recognize the distortion in the DNA helix rather than the specific chemical nature of lesion. On the otherhand, X irradiation produces a lot of single- and double-strand breaks in DNA by the direct action of the radiation. Also, in contrast with the case for UV-irradiation, X-irradiation produces mostly single-base alterations that do not cause large distortions in the DNA helix, and such base damage is recognized individually by enzymes with high specificity.]

Other reports are also consistent with the idea that the DNA damage that leads to spontaneous mutagenesis differs significantly from that produced by X-rays, e.g., the mutations produced in the HGPRT locus of Chinese hamster ovary cells are different for spontaneous and ionizing radiation mutagenesis (Brown and Thacker, 1984), and the levels and types of spontaneous and ionizing radiation-induced base pair changes differ significantly in the *lacI* gene in *E. coli* (Glickman et al., 1980).

However, if one compares data for spontaneous mutagenesis (Schaaper et al., 1986) and for UV-radiation mutagenesis (Schaaper et al., 1987) in wild-type *E. coli*, and normalizes the data so that the number of deletions observed is the same for the two cases, the data indicate that base

substitutions and  $\pm 1$  frameshifts were enhanced about 10-fold by UV irradiation. This suggests that spontaneous base substitutions and  $\pm 1$  frameshifts probably arise from metabolic lesions that are handled by the same mechanisms that handle UV-radiation induced damage. These results also suggest that spontaneous deletions and duplications are probably produced by replication errors or by the repair of spontaneous lesions that are X-ray-like. Therefore, an important project for the future is to determine the relative importance to spontaneous mutagenesis of spontaneous metabolic DNA lesions that are UV-like, and those that are X-ray-like, and to determine their relative importance in producing the different classes of spontaneous mutations.

## VI. Origins of spontaneous mutations

### (1) DNA replication errors

Spontaneous mutagenesis in *E. coli* has both *recA*-dependent and *recA*-independent components (Sargentini and Smith, 1981; and refs. therein), while radiation mutagenesis is exclusively *recA*-dependent (e.g., Witkin, 1976; Sargentini and Smith, 1989; and refs. therein). Replication errors have generally been categorized by their *recA*-independence (reviewed in Cox, 1976), and about half of spontaneous base-substitution mutagenesis is *recA*-independent (Sargentini and Smith, 1981; and refs. therein). A number of different causes of replication errors have been considered over the years.

(a) *Base tautomerization and ionization.* Watson and Crick (1953) postulated that transition mutagenesis (i.e.,  $A \leftrightarrow G$  or  $C \leftrightarrow T$ ) could occur by the production of base tautomers via proton migration. Topal and Fresco (1976) also used tautomerization and base rotation to explain transversion mutagenesis, i.e.,  $(A \text{ or } G) \rightarrow (C \text{ or } T)$ . Sowers et al. (1987) have suggested that ionized base pairs may also contribute to spontaneous mutagenesis.

The basis of these models is that at the time of replication a base in the DNA template or in a nucleoside triphosphate develops an inappropriate coding property, which causes the incorporation of an incorrect base in the nascent DNA.

Although such models are appealing, they are difficult to verify, and certainly do not explain the fact that much of spontaneous mutagenesis is due to errors made during DNA repair (see below).

(b) *Miscoding lesions.* When DNA bases are modified spontaneously, e.g., by deamination, oxidation or methylation, frequently they can pair with a different base than they did before modification (i.e., they are miscoding lesions), leading to the fixation of a mutation during DNA replication.

Since spontaneous cytosine deamination occurs 266-fold more frequently in single-stranded DNA than in double-stranded DNA (reviewed in Billen, 1990), it is not surprising to find that 24 of 31 (77%) of the spontaneous mutations arising from cytosine deamination (i.e., uracil codes for adenine, producing  $G:C \rightarrow A:T$  transitions), occurred in the nontranscribed strand of the *lacI* gene in an *ung* strain of *E. coli* (Fix and Glickman, 1987). [N.B. The nontranscribed strand is considered to be single-stranded during transcription, and the *ung* strain is deficient in uracil DNA glycosylase, an enzyme that removes uracil residues from DNA.]

The spontaneous deamination of cytosine was more frequent ( $\sim 10 \times$ ) at sites for converted suppressor mutations than at sites for de novo suppressor mutations, suggesting that "the physical structure of the DNA in vivo is different at different sites in the seven-tRNA operon" (Bockrath and Mosbaugh, 1986).

When 5-methyl cytosine deaminates, the resulting thymine codes for adenine, and thus will produce a  $G:C \rightarrow A:T$  transition during DNA replication (Duncan and Miller, 1980).

Cytosine deamination also appears to play a major role in spontaneous mutagenesis (APRT locus) in Chinese hamster ovary cells, and the majority of these mutations also occur in the nontranscribed strand (de Jong et al., 1988).

However, coupled with the expected 266-fold greater frequency of spontaneous cytosine deamination in the single-stranded, nontranscribed strand in mammalian cells is the observation that there is the selective repair in active genes of certain types of DNA damage in the transcribed strand relative to the nontranscribed strand in

both mammalian cells and *E. coli* (e.g., Hanawalt, 1987; and a review by Terleth et al., 1991). Such selective repair should reduce the number of mutations in the transcribed strand relative to the nontranscribed strand, assuming that this selective repair is composed of the 'normal' mix of error-free and mutagenic mechanisms.

Methylated guanine is another modified base that may occur spontaneously, and would produce G:C → A:T transitions (reviewed in Drake et al., 1983). However, endogenous methylation does not appear to play a role in spontaneous mutagenesis during growth phase, but it does play a significant role during nongrowth conditions (Section V.4).

When guanine is oxidized to form 8-oxoguanine, it no longer codes for cytosine, rather it codes for adenine, with the resulting formation of G → T transversions (Shibutani et al., 1991). The enzyme that removes 8-oxoguanine from DNA is 8-oxoguanine DNA glycosylase (formerly called fapy glycosylase; Tchou et al., 1991). A strain carrying a mutation in *mutM*, a mutator locus in *E. coli*, shows a 14-fold enhancement in G:C → T:A transversions, with little or no effect on other types of base substitutions (Cabrera et al., 1988). The *mutM* gene codes for 8-oxoguanine DNA glycosylase (Michaels et al., 1991).

(c) *Noncoding lesions.* Noncoding lesions in DNA are usually handled by DNA repair processes (see below), but there are a few examples where such damage is processed by DNA replication. The process for the handling (or mishandling) of noncoding lesions by replication is called translesion synthesis, "a many protein affair" (Echols and Goodman, 1990). One cellular reaction in *E. coli* to replication-blocking lesions is to induce the SOS response, which depends upon the activation of the RecA protein. After the induction of the SOS genes, replication resumes and mutations are produced. There are several models to explain how DNA replication can resume at a blockage: (1) Polymerases are induced (or modified) that have lower fidelity (e.g., Pol II and Pol I\*) (2) RecA protein itself can relax the fidelity of Pol III. In this regard, it is of interest that the overproduction of the  $\epsilon$  subunit (i.e., the editing function) of Pol III counteracts the SOS

mutagenic response of *E. coli* (Jonczyk et al., 1988), and the absence of the  $\epsilon$  subunit (*mutD*) causes a huge increase ( $10^3$  to  $10^5$  times) in spontaneous mutations (Fowler et al., 1986; Piechocki et al., 1986). (3) Random bases are inserted opposite a lesion such as a pyrimidine dimer, but the distortion in the DNA caused by the presence of the lesion does not permit the polymerase to continue chain elongation. The UmuC protein and its SOS-activated partner UmuD' are hypothesized to permit chain elongation past DNA-distorting lesions. However, "the biochemical mechanisms (for these three processes) remain to be established" (reviewed in Echols and Goodman, 1990).

Perhaps the best studied case of translesion synthesis involves AP sites, which are known to be mutagenic (e.g., Schaaper and Loeb, 1981; Loeb and Preston, 1986). While AP sites are clearly noncoding lesions and are subject to repair processes (i.e., AP endonucleases; see Lindahl, 1990), DNA polymerase occasionally handles these lesions simply by introducing an adenine opposite an AP site. In some cases adenine will be the correct base, but in most cases it will not be correct, and will lead to a mutation. dATP is also preferentially inserted opposite a number of bulky chemical adducts to DNA (e.g., aminofluorene, aflatoxin). This so-called 'A rule' of mutagen specificity has been reviewed (Strauss, 1991).

(d) *Polymerase errors.* A number of laboratories have studied the fidelity of various purified DNA polymerases on various purified templates (e.g., Roberts and Kunkel, 1986; Goodman, 1988; Kunkel, 1990; Loeb and Cheng, 1990). In general, the frequencies of base mispairs, base additions and deletions caused by DNA polymerase errors vary widely, and depend both on the base sequence of the template and on the specific DNA polymerase used. The error rate for DNA polymerases in vitro is  $10^{-3}$  to  $10^{-6}$  errors per base pair replicated, versus  $10^{-9}$  to  $10^{-11}$  errors per base pair replicated in vivo. Clearly, cells have additional mechanisms for increasing the accuracy of DNA replication.

If an incorrect nucleotide is incorporated into DNA, and it escapes the editing function of the

DNA polymerase, a second line of defense, *mismatch repair*, is available to a cell to increase the fidelity of DNA replication (e.g., Radman and Wagner, 1986; Modrich, 1987, 1989). In brief, there are several enzymes that recognize mismatched base pairs. Knowing which of the two mismatched bases to cut out is determined in *E. coli* by the presence of methylated bases in the old strand of DNA, and the absence of methylated bases in the newly synthesized strand of DNA. Mutants deficient in methyl-directed mismatch repair (*mutH*, *mutL*, *mutS*) show high spontaneous mutation frequencies (260-fold greater than wild-type cells; Schaaper and Dunn, 1991), but cells deficient in DNA adenine methylation (*dam*) only show a 20-fold increase in spontaneous mutations (Glickman, 1979). Mismatch repair is presumably undirected (i.e., random) in unmethylated DNA (Radman and Wagner, 1986). It is curious that strains with mutations that directly affect the methyl-directed mismatch repair process show much higher spontaneous mutation frequencies than do strains deficient in methylation, suggesting that the methyl-directed pathway may be only one component of the multifaceted *mutHLS*-dependent repair system (Modrich, 1987).

In support of this concept, there is evidence that mismatch repair can also be initiated by DNA-strand breaks (e.g., Claverys and Mejean, 1988; and refs. therein). Therefore, the reduced frequency of spontaneous mutagenesis in a *dam* strain relative to, e.g., a *mutS* strain, may be due to the fact that mismatch repair can still function, but at a reduced efficiency, in a *dam* strain due to the presence of DNA-strand breaks.

In addition, it has been postulated (Glickman and Radman, 1980) and proven (Wang and Smith, 1986) that DNA double-strand breaks are formed in *dam* strains due to the occasional cutting of both strands of DNA during mismatch repair in cells with unmethylated DNA. These extra DNA double-strand breaks in a *dam* strain could also contribute to the strain's higher level of spontaneous mutagenesis versus a wild-type strain (e.g., the repair of DNA double-strand breaks is required for the production of  $\gamma$ -radiation-induced long deletions; Sargentini and Smith, 1991).

Strains deficient in DNA single-strand binding

protein show enhanced (2-fold, *ssb-1*; 10-fold, *ssb-113*) spontaneous mutagenesis. Since the single-strand binding protein enhances replication fidelity and is also required for mismatch repair in vitro, the enhanced spontaneous mutagenesis in *ssb* strains may be due to a combination of these two effects (reviewed in Meyer and Laine, 1990). The single-strand binding protein is also involved in postreplication repair (Sharma and Smith, 1985; and refs. therein), and is required in an in vitro model system for the repair of DNA double-strand breaks (Wang and Smith, 1989).

Imbalances in the nucleotide pools have a marked effect on spontaneous mutagenesis, e.g., mutations (APRT locus) induced in Chinese hamster ovary cells by excess dTTP or dCTP are dominated by the misincorporation of the nucleotide in excess. Also, the shift in mutation spectrum is influenced by the nucleotides surrounding the one that is altered (Phear et al., 1987; see also Kunz, 1982).

The several models for the formation of frameshift mutations by replication errors have been reviewed (Ripley, 1990). These include the Streisinger model for slippage and misalignment between direct repeats (i.e., runs of the same base pairs), and the enhancement of such events by the formation of palindromes and DNA single-strand breaks.

Slippage and mispairing during DNA replication, as the mechanism for the production of *recA*-independent spontaneous long deletions, is discussed below in the section on DNA repair.

## (2) Recombination errors

The available data on the genetic control of spontaneous recombination and of spontaneous mutagenesis (base substitutions) in bacteria and fungi have been compared, and it was concluded that "recombination errors do not play a major role in spontaneous mutagenesis" (Sargentini and Smith, 1985). For example, the *umuC* mutation, which abolishes the error-prone repair-dependent sector of spontaneous mutagenesis (base substitutions), had no effect on recombination (conjugation assay) (Sargentini and Smith, 1981).

However, spontaneous frameshifts and deletions seem to result from mechanisms involving recombination and replication (reviewed in Sar-

gentini and Smith, 1985). Recombination plays a major role in certain DNA-repair processes, e.g., the recombinational repair of ionizing-radiation-induced DNA double-strand breaks is responsible for the formation of long deletion mutations (Sargentini and Smith, 1991). There is a *recA*-dependent mode of excision repair (Smith and Sharma, 1987; and refs. therein), and it is mutagenic (Nishioka and Doudney, 1970; Bridges and Mottershead, 1971). Since *uvr*<sup>-</sup> strains show more spontaneous mutagenesis than do wild-type cells (reviewed in Sargentini and Smith, 1981), this suggests that postreplication repair is more mutagenic than is excision repair. Postreplication repair is also *recA*-dependent, and is a multipathway process for the repair of daughter-strand gaps and of the DNA double-strand breaks that can arise at these gaps (reviewed in Smith and Wang, 1989). Therefore, recombination plays an important role in several types of mutagenic DNA repair.

### (3) Repair errors

Mutations that sensitize cells to DNA-damaging agents have been shown to do so by reducing the ability of the cells to repair DNA damage. The concept of error-prone (mutagenic) DNA repair resulted from the finding by Witkin (1967) that a *lexA(exrA)* strain was not only more sensitive to killing by UV irradiation, but was also deficient in UV-radiation mutagenesis. The *lexA* gene product is the repressor for the *recA* gene, a gene that is required for both UV- and X-radiation mutagenesis, and is required for about 50% of spontaneous base-substitution mutagenesis (see Sargentini and Smith, 1981, 1989; and refs. therein).

As mentioned above, the part of spontaneous mutagenesis that is *recA*-independent is considered to be due to replication errors (both intrinsic errors and those due to the replication of damaged DNA), while the *recA*-dependent part of spontaneous mutagenesis is considered to be due to DNA-repair errors.

When bacterial mutations affecting DNA repair were listed and their effects on spontaneous and induced mutagenesis were tabulated (Sargentini and Smith, 1985), in general, those mutations that enhance UV-radiation mutagenesis

(e.g., *uvrABC*, *polA*, *lig*, *recF*) also enhance spontaneous mutagenesis, and those that reduce UV-radiation mutagenesis (e.g., *lexA*, *recA*, *umuCD*, *polC(Ts)*, *uvrD*) also reduce spontaneous mutagenesis. These observations led to the conclusion that spontaneous mutagenesis is due to the metabolic production of 'UV-like' damage rather than 'X-ray-like' damage since, e.g., the *uvrABC* mutations have little effect on survival or mutagenesis after X- or  $\gamma$ -irradiation (Sargentini and Smith, 1981, 1985; Smith and Sargentini, 1985).

However, Cunningham et al. (1986) reported that an *xth nth nfo* strain of *E. coli* showed a spontaneous mutation frequency that was 5-fold greater than that for the wild-type strain. The *xth nth nfo* strain is deficient in three enzymes for the repair of damaged bases and/or AP sites, lesions commonly produced by X irradiation. Furthermore, the *xth nth nfo* strain is hypermutable by  $\gamma$  irradiation (N.J. Sargentini and K.C. Smith, unpublished observation).

The most logical conclusion from these data is that spontaneous mutagenesis is produced by metabolic events that result in the production both of 'UV-like' and 'X-ray-like' damage (see also Section V.6). Which of these two types of damage is the most important for spontaneous mutagenesis is a question that can never be answered in *general terms*, since the answer will depend upon the experimental conditions, the genetic background of the cells being studied, and the type of assay being used.

From a survey of the literature, it was concluded that about 50% of the spontaneous base substitutions in *E. coli* and perhaps 90% in *Saccharomyces cerevesiae* are due to mutagenic DNA repair. However, spontaneous frameshifts and deletions seem to result from mechanisms involving recombination and replication (Sargentini and Smith, 1985).

There are more data in the literature on spontaneous base substitution mutagenesis, because this type of mutation has been easier to study. Recently, a convenient assay has been developed for the study of long deletion mutagenesis (Albertini et al., 1982). In this assay, spontaneous deletions in a *lacI-Z* fusion ranging from 700 to 1000 base pairs were measured and sequenced.

Deletions of this size occur almost exclusively at short repeated sequences in both *recA*<sup>+</sup> and *recA*<sup>-</sup> strains, but were detected 25-fold more frequently in the *recA*<sup>+</sup> background.

Using this assay, it was shown that  $\gamma$ -radiation-induced long deletions were totally dependent upon the *recB*-dependent (but not the *recF*-dependent) recombinational repair of DNA double-strand breaks, but spontaneous deletions were only reduced about 3-fold by a *recB* mutation (Sargentini and Smith, 1991). This difference of a 3-fold reduction for *recB* (Sargentini and Smith, 1991) and 25-fold reduction for *recA* (Albertini et al., 1982) is probably due to experimental differences, however, the most important fact is that neither a *recA* or a *recB* mutation eliminates all of spontaneous deletion mutagenesis. Therefore, the conclusion must be that *recA* *recB*-dependent spontaneous long-deletion mutagenesis is due to the recombinational repair of DNA double-strand breaks produced metabolically, while the *recA* *recB*-independent production of long deletions is probably due to "slippage and mispairing" during DNA replication (e.g., Ripley, 1990). Support for this concept comes from the work of Kunkel (1985), who showed that deletions arise between repetitive DNA sequences in an in vitro DNA synthesis system that requires only eucaryotic DNA polymerase  $\beta$ . DNA synthesis on the leading and lagging strands contribute equivalently to the deletion process by replication slippage (Weston-Hafer and Berg, 1991).

## VII. Random versus directed mutagenesis

From our knowledge about the many different DNA-repair systems, several of which can function as backup systems for other repair systems, and of the extreme importance of maintaining the integrity of a cell's genome, the concept of mutagenic repair seems at first to be inconsistent with nature. However, since evolution is "a process of continuous change from a lower, simpler, or worse to a higher, more complex or better state" (Webster's New Collegiate Dictionary, 1977), it is obvious that if errors were not made during DNA repair, replication, and recombination there would be no mutations, and therefore, no evolu-

tion. Several papers have appeared recently discussing the role of mutations in evolution e.g., Arber, 1990, 1991; Drake, 1991b; Wintersberger, 1991, and additional references are cited below.

Most spontaneous mutagenesis experiments are performed upon cells in exponential growth, a far cry from the situation for organisms outside of the laboratory where "normal" evolution occurs. Since the spontaneous mutation frequency of an organism is the resultant of all of those normal growth and environmental factors that are mutagenic, and all of those normal growth and environmental factors that are antimutagenic, any change in experimental protocol that upsets the balance between these mutagenic and antimutagenic forces will modify the spontaneous mutation frequency of the organism, but this change in mutation frequency will not be uniform for all genes or for all types of mutations [e.g., active genes are more susceptible to damage than are inactive genes (Davis, 1989), and are more susceptible to spontaneous mutagenesis (Korogodin et al., 1991)].

In general, spontaneous mutations are considered to be produced randomly, and without respect to any advantage that they might offer to the cell. However, based upon data for cells not in exponential growth that do not strictly fit the statistical criterion of randomness, Cairns et al. (1988) proposed that "cells may have mechanisms for choosing which mutations will occur". Subsequently, a number of papers have been published to debate this concept of self 'directed' mutagenesis for cells under stress. A recent article in *BioScience*, entitled "Can organisms direct their evolution?" (Gillis, 1991), attempts to put this controversy into perspective.

In this article Dr. Bruce Levin, an evolutionary biologist, is quoted as saying that "By itself, a deviation from the Luria-Delbruck distribution of mutations in a fluctuation test is not sufficient evidence for directed mutation." In support of this conclusion, Stewart et al. (1990) have shown that growth rate and plating efficiencies can cause such deviations from apparent randomness.

It is appropriate to call to the reader's attention another era in biology when mathematical models were accepted as gospel, and biological mechanisms were ascribed to graphical variables

as proven. "Target theory" in radiation biology was devised long before DNA repair was known (e.g., Lea, 1962). Cells were considered to contain physical targets that were inactivated by the actions of radiation. In some cells a single "hit" by the radiation in a single "target" appeared sufficient for inactivation. In other cells, multi-hits in multi-targets apparently were required, and so on. The shapes of radiation-survival curves were fit to mathematical formulas, and the graphical variables (e.g.,  $n$ ,  $D_q$ ,  $D_0$ ,  $D_{37}$ ) were assigned biological meaning without proof. Seemingly endless papers have been published over the years refining target theory to accommodate cases where the biology didn't fit the previous mathematics. Fortunately, there have also been papers showing that target theory by itself cannot define biological mechanisms. The point is that an immense amount of money and time was wasted (as it turned out) by people who have tried to make curve fitting explain biology.

There is the same potential for a waste of time and effort over the "Cairnsian" (Hall, 1990) model of directed mutagenesis (Cairns et al., 1988), which is based upon a small deviation from a mathematical model by Luria and Delbruck (1943) that was modified by Lea and Coulson (1949), and "refinements" of this mathematical model continue to appear (e.g., Lenski et al., 1989; Stewart et al., 1990; Koziol, 1991).

Fortunately, other people see the hazards of ascribing complicated biological mechanisms to minor variations in statistics. For example, although the data of Hall (1990) confirm the data of Cairns et al. (1988) in showing that reversions sometimes occur more frequently when advantageous than when neutral, he cautions that such results "can be explained on the basis of underlying random mechanisms that act during prolonged periods of physiological stress, and that 'directed' mutations are not necessarily the basis of those observations".

Even Cairns et al. (1988) state that "The shape of the distribution of Lac<sup>+</sup> revertants of strains bearing an amber mutation in *lacZ* depends upon the genotype of the strain and on the medium in which it was growing before being plated." There are other results in the paper by Cairns et al. (1988) that should have prevented the formula-

tion of the 'directed' mutagenesis hypothesis. These authors state that "The *uvrB* strain produces each day about 3–5 times as many late mutants as the *uvr*<sup>+</sup> strain and it is therefore the strain of choice when testing for the possible existence of directed mutations." These results for *E. coli uvrB* tell one immediately that the hypothetical phenomenon of directed mutagenesis is under genetic control, and is enhanced by the absence of excision repair, with the consequent necessity for the cell to deal with spontaneous DNA lesions by replication and by postreplication repair, which is a slow process compared to excision repair, and is more mutagenic.

"It is the guiding principle in science that a radical new interpretation (in this case, one invoking the inheritance of acquired characteristics) should only be considered if simpler explanations based on existing knowledge are inadequate" (Holliday and Rosenberger, 1988).

One can only hope that the "directed mutagenesis theory" will not result in as much wasted time and money as has the "target theory" of radiation biology. This is not to say that directed mutagenesis may not exist, it just means that its existence will not be proven by statistics or by ignoring the fact that spontaneous mutagenesis in different genes can be differentially affected both qualitatively and quantitatively by subtle variations in experimental protocols. In fact, learning more about the factors that selectively affect spontaneous mutagenesis in different genes is the real challenge for future work on spontaneous mutagenesis.

The observations that spontaneous mutations occur more often when advantageous than when neutral (e.g., Hall, 1990, 1991) suggest that studies on the genetic control and molecular mechanisms of spontaneous mutagenesis (e.g., Table 1), especially as they apply to studies on evolution, should be performed on cells under stressed conditions rather than under "ideal" growth conditions. [Note added in revision: The new paper by Cairns and Foster (1991) abandons the term "directed mutagenesis" in favor of the more moderate term "adaptive mutation", but this paper does not alter my conclusion that a better understanding of the factors that affect spontaneous



mutagenesis would preclude the need even for the concept of “adaptive mutation”.]

### VIII. Spontaneous carcinogenesis

“Both cancer and ageing might be the somatic ‘price’ individual organisms have to pay for the germinal mutations which could contribute to the survival of the species” (Trosko and Chang, 1976).

There are probably as many theories on the origin of spontaneous carcinogenesis as there are authors who have written about the subject, but it is not within the scope of this review to discuss all of these theories. What I wish to emphasize, however, is that the concept that spontaneous mutagenesis is the “net result of all that can go wrong with DNA during the life cycle of an organism” (Glickman et al., 1986) applies equally

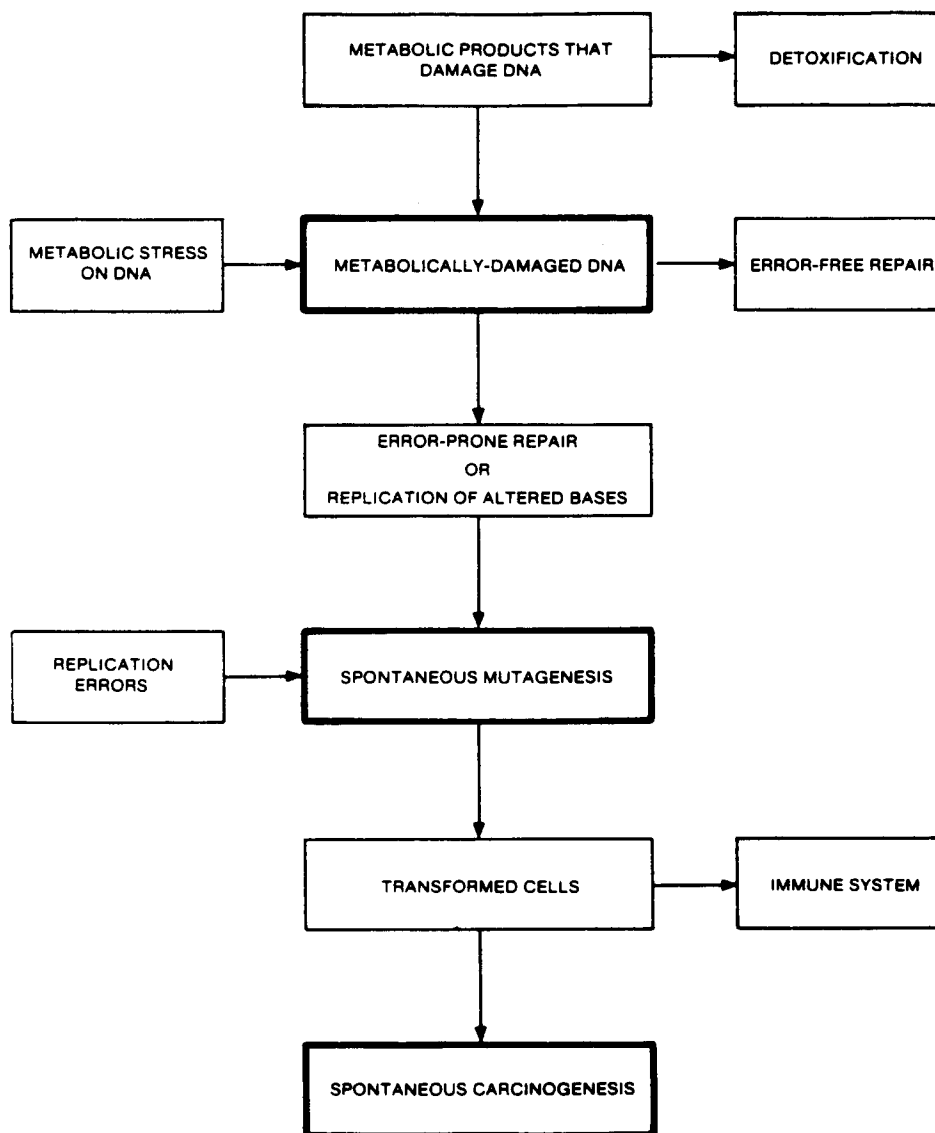


Fig. 1. Metabolic mechanisms both for the initiation and for the avoidance of spontaneous mutagenesis and spontaneous carcinogenesis (from Smith and Sargentini, 1986).

well to spontaneous carcinogenesis. The notion of normal metabolism being a major source of spontaneous mutagenesis (e.g., Sargentini and Smith, 1981) is now also being considered as the major source of spontaneous carcinogenesis, especially for those human cancers that occur at similar incidences among individuals of diverse genetic backgrounds living in different geographical areas (e.g., Loeb, 1989, 1991; Rudiger, 1990).

Thus, the susceptibility of a person to spontaneous carcinogenesis is the net result of that person's natural defenses against normal metabolic products and environmental agents that can damage DNA, the ability of his/her cells to repair accurately such damage to DNA, and the ability of his/her immune system to neutralize any transformed cells that may result from spontaneous mutagenesis. These concepts are diagrammed in Fig. 1.

Therefore, people predisposed to cancer can be overproducers of agents that damage DNA, or can be underproducers of products that detoxify these agents. They can be deficient in error-free DNA repair, replication and recombination, or they can be immunologically deficient, or a combination of these factors.

Bloom's syndrome serves as an example of a case where a person is either an overproducer of agents that damage DNA, or an underproducer of products that detoxify these agents. In a mixed culture of normal human blood lymphocytes and fibroblasts from a patient with Bloom's syndrome, the fibroblasts from the patient with Bloom's syndrome produce a clastogenic factor that causes chromosomal aberrations in the normal human blood lymphocytes, while fibroblasts from normal individuals have no such effect (Emerit and Cerutti, 1981). Bloom's syndrome cells also show an enhanced frequency of spontaneous mutagenesis (Warren et al., 1981), and patients with Bloom's syndrome show an increased incidence of cancer (Friedberg, 1985).

The xeroderma pigmentosum (XP) syndrome serves as an example where patients are deficient in one type of error-free repair, and thus show enhanced UV-radiation mutagenesis and susceptibility to sunlight-induced skin cancer (Friedberg et al., 1979). While data on spontaneous mutagenesis are not available for XP cells, it is of inter-

est that XP individuals are more prone to certain types of internal neoplasia (Kraemer et al., 1984).

Since spontaneous carcinogenesis is just another phenotype of spontaneous mutagenesis, if we are to better understand the mechanisms of spontaneous carcinogenesis, we need a better understanding of the molecular mechanisms of spontaneous mutagenesis, and of the subtle experimental variables that affect spontaneous mutagenesis.

## IX. Future directions

Spontaneous mutagenesis is the most important area of the science of mutagenesis, because it is probably the most important factor in evolution, aging and spontaneous carcinogenesis, yet it has not received the careful scientific attention that it deserves.

Furthermore, many scientists seem to be unaware that the spontaneous mutation frequency measured in a particular system depends upon many subtle and often ignored experimental parameters. The following experimental parameters have been discussed herein.

- (1) Logarithmic growth vs. stationary phase (or growth limited).
- (2) Rich growth medium versus minimal medium in logarithmic growth.
- (3) Composition of the growth medium, e.g., nucleotide pool imbalances; mutagens (e.g., phenylalanine); antimutagens (e.g., cystine).
- (4) Growth rate.
- (5) Aeration rate.
- (6) Purity of the agar (i.e., presence of DNA-repair inhibitors).
- (7) Laboratory lighting (for both the cultures and for stored media).
- (8) Genetic background (see Table 1).
- (9) The presence of insertion elements.
- (10) The type of mutation assay used.
- (11) The locus being studied.
- (12) An active gene versus an inactive gene.
- (13) Is the spontaneous lesion located before or after the replication fork?

This is probably only a partial list of the factors that affect spontaneous mutagenesis, since I may have missed some important papers in the

literature, and I am certain that other factors affecting spontaneous mutagenesis will be discovered in the future.

Even with our present state of knowledge, it makes no sense to compare the mutational spectra for cells when the spontaneous data are for exponentially growing cells and the UV-radiation studies are on stationary phase cells (Lee et al., 1988). These authors demonstrated the predictable result that the "A-rule" did not apply for cells that could not synthesize DNA, but did apply for cells in logarithmic growth.

Clearly, there needs to be some general agreement in the scientific community on how best to determine spontaneous mutation frequencies, and how best to report them so that the data will have lasting value. Ideally, one set of isogenic strains carrying mutations affecting spontaneous mutagenesis should be tested under one standardized set of experimental conditions and one set of assays so that the relative contributions of these different genes to spontaneous mutagenesis can be quantitated.

Where different mutation assays must be used, there should be some conversion factor to help normalize the data, e.g., in one set of experiments reported in Section IV.4, spontaneous *trpE9777* (fs) → Trp<sup>+</sup> reversions occurred less than one-third as frequently as did *lacZ53*(UAG) → Lac<sup>+</sup> reversions in the same cells.

In addition to a greater effort toward understanding the molecular mechanisms for spontaneous mutagenesis and the factors that affect these mechanisms, there also needs to be a greater effort toward finding ways to reduce spontaneous mutagenesis, and therefore, to reduce the incidence of spontaneous carcinogenesis and the rate of aging.

Antimutagenesis is an active area of science (e.g., Shankel et al., 1986) but most of these studies are concerned with agents that intercept mutagens and inactivate them before they can do damage (e.g., Hartman and Shankel, 1990). This is an important area of concern for exogenous mutagens such as those that normally appear in our foodstuffs, however, the area of antimutagenesis that needs even more emphasis is to determine how to modulate normal metabolic pathways to minimize the metabolic production of

mutagens, and how to minimize the occurrence of mutagenic DNA replication, repair and recombination.

One approach to this problem has been to emphasize the antimutagenic potential of antioxidants to neutralize the potential mutagenic side effects of normal oxidative metabolism (e.g., Ames and Gold, 1991).

As an example of another approach to this problem, some years ago we reported that spontaneous mutagenesis appeared to be due to the metabolic production of DNA damage that was "UV-like" rather than "X-ray-like". This prompted a poster at an antimutagenesis meeting (Shankel et al., 1986; p. 580) entitled "The Ideal Antimutagen May be a Quencher of Excited States".

Since rare earth metals are good quenchers of excited states, I tried lanthanum as an inhibitor of spontaneous mutagenesis. Lanthanum acetate was nontoxic to *E. coli uvrB* (SR1091) at 1 mg/ml (3.2 mM), and decreased spontaneous mutagenesis (*lacZ53* → Lac<sup>+</sup>) about 5-fold. Although lanthanum is a good quencher of excited states, it is also an antagonist of calcium, and calcium is important in many areas of metabolism. Which of these two mechanisms is the basis for the effectiveness of lanthanum in reducing spontaneous mutagenesis is not yet known. However, the lanthanum experiment serves as an example of an approach to modulate normal metabolic reactions to reduce the metabolic production of normal mutagens and/or to inhibit mutagenic DNA repair, replication and recombination.

While there is current interest and activity toward reducing the intake of foods that are mutagenic and for increasing the intake of foods that are antimutagenic, there needs to be more activity directed toward finding diet supplements that can safely inhibit mutagenic DNA repair and replication, or that can reduce the production of mutagenic agents by normal metabolism. I don't propose a shotgun approach to testing chemicals off the shelf, rather, a systematic approach to understanding the many factors that affect spontaneous mutagenesis will provide information for selecting such useful chemicals. Two reviews have appeared that focus on antimutagenesis (Kuroda and Inoue, 1988) and anticarcinogenesis (Booth-

man et al., 1988) by factors that affect DNA repair.

## X. Acknowledgements

I wish to thank Drs. Robert G. Fowler, Arthur P. Grollman, Tzu-chien V. Wang and Neil J. Sargentini for valuable discussions, and for critical comments on this manuscript.

This work was supported by U.S. Public Health Service grant CA33738 from the National Cancer Institute, DHHS.

## Note added in proof

In a recent paper, J.A. Halliday and B.W. Glickman (Mechanisms of spontaneous mutation in DNA repair-proficient *Escherichia coli*, *Mutation Res.*, 250 (1991) 55–71) have considered a number of mutational mechanisms to “help visualize the origin of each class of (spontaneous) mutation” in the *lacI* gene (729 sequenced independent mutations). “One common factor in all classes of events is the influence on their frequency of occurrence of specific sequences which have been directly implicated as sites of protein:DNA recognition or display a recognisable homology to such sequences.” This is a very exciting observation, and adds yet another parameter to the long list presented in this review of the factors that influence spontaneous mutagenesis.

## References

- Albertini, A.M., M. Hofer, M.P. Calos and J.H. Miller (1982) On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions, *Cell*, 29, 319–328.
- Ames, B.N. (1983) Dietary carcinogens and anticarcinogens, Oxygen radicals and degenerative diseases, *Science*, 221, 1256–1264.
- Ames, B.N., and L.S. Gold (1991) Endogenous mutagens and the causes of aging and cancer, *Mutation Res.*, 250, 3–16.
- Ames, B.N., M. Profet and L.S. Gold (1990) Dietary pesticides (99.99% all natural), *Proc. Natl. Acad. Sci. (U.S.A.)*, 87, 7777–7781.
- Arber, W. (1990) Mechanisms in microbial evolution, *J. Struct. Biol.* 104, 107–111.
- Arber, W. (1991) Elements in microbial evolution, *J. Mol. Evol.*, 33, 4–12.
- Au, K.G., S. Clark, J.H. Miller and P. Modrich (1989) *Escherichia coli mutY* gene encodes an adenine glycosylase active on G-A mispairs, *Proc. Natl. Acad. Sci. (U.S.A.)*, 86, 8877–8881.
- Barknecht, T.R., and K.C. Smith (1977) Ultraviolet radiation-induced mutability of isogenic *uvrA* and *uvrB* strains of *Escherichia coli* K-12 W3110, *Photochem. Photobiol.*, 26, 643–645.
- Bhatnagar, S.K., and M.J. Bessman (1988) Studies on the mutator gene, *mutT* of *Escherichia coli*, Molecular cloning of the gene, purification of the gene product, and identification of a novel nucleoside triphosphatase, *J. Biol. Chem.*, 263, 8953–8957.
- Billen, D. (1990) Spontaneous DNA damage and its significance for the ‘negligible dose’ controversy in radiation protection, *Radiation Res.*, 124, 242–245.
- Bockrath, R., and P. Mosbaugh (1986) Mutation probe of gene structure in *E. coli*: suppressor mutations in the seven-tRNA operon, *Mol. Gen. Genet.*, 204, 457–462.
- Boothman, D.A., R. Schlegel and A.B. Pardee (1988) Anticarcinogenic potential of DNA-repair modulators, *Mutation Res.*, 202, 393–411.
- Breimer, L.H. (1990) Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: the role of DNA base damage, *Mol. Carcinogen.*, 3, 188–197.
- Bridges, B.A., and R. Mottershead (1971) RecA<sup>+</sup>-dependent mutagenesis occurring before DNA replication in UV- and  $\gamma$ -irradiated *Escherichia coli*, *Mutation Res.*, 13, 1–8.
- Brown, R., and J. Thacker (1984) The nature of mutants induced by ionising radiation in cultured hamster cells, I. Isolation and initial characterisation of spontaneous, ionising radiation-induced, and ethyl methanesulphonate-induced mutants resistant to 6-thioguanine, *Mutation Res.*, 129, 269–281.
- Cabrera, M., Y. Nghiem and J.H. Miller (1988) *mutM*, a second mutator locus in *Escherichia coli* that generates G·C → T·A transversions, *J. Bacteriol.*, 170, 5405–5407.
- Cadenas, E. (1984) Biological chemiluminescence, *Photochem. Photobiol.*, 40, 823–830.
- Cadenas, E., H. Sies, A. Campa and G. Cilento (1984) Electronically excited states in microsomal membranes: use of chlorophyll-*a* as an indicator of triplet carbonyls, *Photochem. Photobiol.*, 40, 661–666.
- Cairns, J., and P.I. Foster (1991) Adaptive reversion of a frameshift mutation in *Escherichia coli*, *Genetics*, 128, 695–701.
- Cairns, J., J. Overbaugh and S. Miller (1988) The origin of mutants, *Nature (London)*, 335, 142–145.
- Choe, M., and W.S. Reznikoff (1991) Anaerobically expressed *Escherichia coli* genes identified by operon fusion techniques, *J. Bacteriol.*, 173, 6139–6146.
- Cilento, G. (1980) Photochemistry in the dark, *Photochem. Photobiol. Rev.*, 5, 199–228.
- Cilento, G., and W. Adam (1988) Photochemistry and photobiology without light, *Photochem. Photobiol.*, 48, 361–368.
- Claverys, J.-P., and V. Mejean (1988) Strand targeting signal(s) for in vivo mutation avoidance by post-replication mismatch repair in *Escherichia coli*, *Mol. Gen. Genet.*, 214, 574–578.

- Cox, E.C. (1976) Bacterial mutator genes and the control of spontaneous mutagenesis, *Annu. Rev. Genet.*, 10, 135–156.
- Cunningham, R.P., S.M. Saporito, S.G. Spitzer and B. Weiss (1986) Endonuclease IV (*nfo*) mutant of *Escherichia coli*, *J. Bacteriol.*, 168, 1120–1127.
- Davis, B.D. (1989) Transcriptional bias: A non-Lamarckian mechanism for substrate-induced mutations, *Proc. Natl. Acad. Sci. (U.S.A.)*, 86, 5005–5009.
- de Jong, P.J., A.J. Grosovsky and B.W. Glickman (1988) Spectrum of spontaneous mutation at the *APRT* locus of Chinese hamster ovary cells: an analysis at the DNA sequence level, *Proc. Natl. Acad. Sci. (U.S.A.)*, 85, 3499–3503.
- DeMarini, D.M., H.E. Brockman, F.J. de Serres, H.H. Evans, L.F. Stankowski Jr. and A.B. Hsie (1989) Specific-locus mutations induced in eukaryotes (especially mammalian cells) by radiation and chemicals: a perspective, *Mutation Res.*, 220, 11–29.
- Diver, W.P., N.J. Sargentini and K.C. Smith (1982) A mutation (*radA100*) in *Escherichia coli* that selectively sensitizes cells grown in rich medium to X- or U.V.-radiation, or methyl methanesulphonate. *Int. J. Radiat. Biol.*, 42, 339–346.
- Drake, J.W. (1991a) Spontaneous mutation, *Annu. Rev. Genet.*, 25, 125–146.
- Drake, J.W. (1991b) A constant rate of spontaneous mutation in DNA-based microbes, *Proc. Natl. Acad. Sci. (U.S.A.)*, 88, 7160–7164.
- Drake, J.W., B.W. Glickman and L.S. Ripley (1983) Updating the theory of mutation, *Am. Sci.*, 71, 621–630.
- Drobetsky, E.A., A.J. Grosovsky and B.W. Glickman (1987) The specificity of UV-induced mutations at an endogenous locus in mammalian cells, *Proc. Natl. Acad. Sci. (U.S.A.)*, 84, 9103–9107.
- Duncan, B.K., and J.H. Miller (1980) Mutagenic deamination of cytosine residues in DNA, *Nature (London)*, 287, 560–561.
- Echols, H., and M.F. Goodman (1990) Mutation induced by DNA damage: a many protein affair, *Mutation Res.*, 236, 301–311.
- Emerit, I., and P. Cerutti (1981) Clastogenic activity from Bloom syndrome fibroblast cultures, *Proc. Natl. Acad. Sci. (U.S.A.)*, 78, 1868–1872.
- Erlich, H.A., and E.C. Cox (1980) Interaction of an *Escherichia coli* mutator gene with a deoxyribonucleotide effector, *Mol. Gen. Genet.*, 178, 703–708.
- Farr, S.B., R. D'Ari and D. Touati (1986) Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase, *Proc. Natl. Acad. Sci. (U.S.A.)*, 83, 8268–8272.
- Fix, D.F., and B.W. Glickman (1987) Asymmetric cytosine deamination revealed by spontaneous mutational specificity in an  $\text{Ung}^-$  strain of *Escherichia coli*, *Mol. Gen. Genet.*, 209, 78–82.
- Fix, D.F., P.A. Burns and B.W. Glickman (1987) DNA sequence analysis of spontaneous mutation in a PolA1 strain of *Escherichia coli* indicates sequence-specific effects, *Mol. Gen. Genet.*, 207, 267–272.
- Fowler, R.G., R.M. Schaaper and B.W. Glickman (1986) Characterization of mutational specificity within the *lacI* gene for a *mutD5* mutator strain of *Escherichia coli* defective in 3' → 5' exonuclease (proofreading) activity, *J. Bacteriol.*, 167, 130–137.
- Friedberg, E.C. (1985) "DNA Repair", Freeman New York.
- Friedberg, E.C., U.K. Ehmann and J.I. Williams (1979) Human diseases associated with defective DNA repair, *Adv. Radiat. Biol.*, 8, 85–174.
- Friesen, B.S., P.S. Iyer, J.E. Baptist, R. Meyn and J.-M. Rodgers (1970) Glucose-induced resistance to gamma-rays in *Escherichia coli*, *Int. J. Radiat. Biol.*, 18, 159–172.
- Geiger, J.R., and J.F. Speyer (1977) A conditional antimutator in *E. coli*, *Mol. Gen. Genet.*, 153, 87–97.
- Gillis, A.M. (1991) Can organisms direct their evolution? *BioScience*, 41, 202–205.
- Giroux, C.N., J.R.A. Mis, M.K. Pierce, S.E. Kohalmi and B.A. Kunz (1988) DNA sequence analysis of spontaneous mutations in the *SUP4-o* gene of *Saccharomyces cerevisiae*, *Mol. Cell. Biol.*, 8, 978–981.
- Glatt, H. (1990) Endogenous mutagens derived from amino acids, *Mutation Res.*, 238, 235–243.
- Glickman, B.W. (1979) Spontaneous mutagenesis in *Escherichia coli* strains lacking 6-methyladenine residues in their DNA, *Mutation Res.*, 61, 153–162.
- Glickman, B.W., and M. Radman (1980) *Escherichia coli* mutator mutants deficient in methylation-instructed DNA mismatch correction, *Proc. Natl. Acad. Sci. (U.S.A.)*, 77, 1063–1067.
- Glickman, B.W., P.A. Burns and D.F. Fix (1986) Mechanisms of spontaneous mutagenesis: clues from altered mutational specificity in DNA repair-defective strains, in: D.M. Shankel, P.E. Hartman, T. Kada and A. Hollaender (Eds.), *Antimutagenesis and Anticarcinogenesis Mechanisms*, Plenum, New York, pp. 259–281.
- Glickman, B.W., K. Rietveld and C.S. Aaron (1980)  $\gamma$ -Ray-induced mutational spectrum in the *lacI* gene of *Escherichia coli*, Comparison of induced and spontaneous spectra at the molecular level, *Mutation Res.*, 69, 1–12.
- Goodman, M.F. (1988) DNA replication fidelity: kinetics and thermodynamics, *Mutation Res.*, 200, 11–20.
- Gordon, A.J.E., J.A. Halliday, M.J. Horsfall and B.W. Glickman (1991) Spontaneous and 9-aminoacridine-induced frameshift mutagenesis: second-site frameshift mutation within the N-terminal region of the *lacI* gene of *Escherichia coli*, *Mol. Gen. Genet.*, 227, 160–164.
- Gralla, E.B., and J.S. Valentine (1991) Null mutants of *Saccharomyces cerevisiae* Cu, Zn superoxide dismutase: Characterization and spontaneous mutation rates, *J. Bacteriol.*, 173, 5918–5920.
- Grivell, A.R., M.B. Grivell and P.C. Hanawalt (1975) Turnover in bacterial DNA containing thymine or 5-bromouracil, *J. Mol. Biol.*, 98, 219–233.
- Groat, R.G., J.E. Schultz, E. Zychlinsky, A. Bockman and A. Matin (1986) Starvation proteins in *Escherichia coli*: kinetics of synthesis and role in starvation survival, *J. Bacteriol.*, 168, 486–493.
- Hall, B.G. (1990) Spontaneous point mutations that occur more often when advantageous than when neutral, *Genetics*, 126, 5–16.
- Hall, B.G. (1991) Adaptive evolution that requires multiple

- spontaneous mutations: mutations involving base substitutions, Proc. Natl. Acad. Sci. (U.S.A.), 88, 5882–5886.
- Hall, R.M., and W.J. Brammar (1973) Increased spontaneous mutation rates in mutants of *E. coli* with altered DNA polymerase III, Mol. Gen. Genet., 121, 271–276.
- Hanawalt, P.C. (1987) Preferential DNA repair in expressed genes, Environ. Health Perspect., 76, 9–14.
- Hartman, P.E., and D.M. Shankel (1990) Antimutagens and anticarcinogens: a survey of putative interceptor molecules, Environ. Mol. Mutagen., 15, 145–182.
- Hartman, P.E., Z. Hartman, R.C. Stahl and B.N. Ames (1971) Classification and mapping of spontaneous and induced mutations in the histidine operon of Salmonella, Adv. Genet., 16, 1–34.
- Hartman, Z., P.E. Hartman, W.M. Barnes and E. Tuley (1984) Spontaneous mutation frequencies in Salmonella: enhancement of G/C to A/T transitions and depression of deletion and frameshift mutation frequencies afforded by anoxic incubation, Environ. Mutagen., 6, 633–650.
- Hartman, Z., P.E. Hartman and W.L. McDermott (1991) Mutagenicity of cool white fluorescent light for Salmonella, Mutation Res., 260, 25–38.
- Holliday, R., and R.F. Rosenberger (1988) Letter to the Editor, Nature (London), 336, 526.
- Jankovic, M., T. Kostic and D.J. Savic (1990) DNA sequence analysis of spontaneous histidine mutations in a *polAI* strain of *Escherichia coli* K12 suggests a specific role for the GTGG sequence, Mol. Gen. Genet., 223, 481–486.
- Jeggo, P. (1979) Isolation and characterization of *Escherichia coli* K-12 mutants unable to induce the adaptive response to simple alkylating agents, J. Bacteriol., 139, 783–791.
- Jonczyk, P., I. Fijalkowska and Z. Ciesla (1988) Overproduction of the  $\epsilon$  subunit of DNA polymerase III counteracts the SOS mutagenic response of *Escherichia coli*, Proc. Natl. Acad. Sci. (U.S.A.), 85, 9124–9127.
- Kelley, M., and J.M. Baden (1980) Oxygen mutagenicity, Mutation Res., 77, 185–188.
- Kirkwood, T.B.L. (1989) DNA, mutations and aging, Mutation Res., 219, 1–7.
- Kondo, S. (1973) Evidence that mutations are induced by errors in repair and replication, Genetics, Suppl. 73, 109–122.
- Kondo, S., H. Ichikawa, K. Iwo and T. Kato (1970) Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities, Genetics, 66, 187–217.
- Korogodin, V.I., V.L. Korogodina, Cs. Fajsz, A.I. Chepurinov, N. Mikhova-Tsenova and N.V. Simonyan (1991) On the dependence of spontaneous mutation rates on the functional state of genes, Yeast, 7, 105–117.
- Kozioł, J.A. (1991) A note on efficient estimation of mutation rates using Luria-Delbrück fluctuation analysis, Mutation Res., 249, 275–280.
- Kraemer, K.H., M.M. Lee and J. Scotto (1984) DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum, Carcinogenesis, 5, 511–514.
- Kunkel, T.A. (1985) The mutational specificity of DNA polymerase- $\beta$  during in vitro DNA synthesis, Production of frameshift, base substitution, and deletion mutations, J. Biol. Chem., 260, 5787–5796.
- Kunkel, T.A. (1990) Misalignment-mediated DNA synthesis errors, Biochemistry, 29, 8003–8011.
- Kunz, B.A. (1982) Genetic effects of deoxyribonucleotide pool imbalances, Environ. Mutagen., 4, 695–725.
- Kuroda, Y., and T. Inoue (1988) Antimutagenesis by factors affecting DNA repair in bacteria, Mutation Res., 202, 387–391.
- Lawrence, C.W. (1982) Mutagenesis in *Saccharomyces cerevisiae*, Adv. Genet., 21, 173–254.
- Lea, D.E. (1962) Actions of Radiation on Living Cells, (reprinting of the 2nd edn. of 1955), Cambridge University Press, Cambridge, England.
- Lea, D.E., and C.A. Coulson (1949) The distribution of the numbers of mutants in bacterial populations, J. Genet., 49, 264–285.
- Lee, A.T., and A. Cerami (1990) In vitro and in vivo reactions of nucleic acids with reducing sugars, Mutation Res., 238, 185–191.
- Lee, G.S.-F., E.A. Savage, R.G. Ritzel and R.C. von Borstel (1988) The base-alteration spectrum of spontaneous and ultraviolet radiation-induced forward mutations in the *URA3* locus of *Saccharomyces cerevisiae*, Mol. Gen. Genet., 214, 396–404.
- Lenski, R.E., M. Slatkin and F.J. Ayala (1989) Mutation and selection in bacterial populations: alternatives to the hypothesis of directed mutation, Proc. Natl. Acad. Sci. (U.S.A.), 86, 2775–2778.
- Lindahl, T. (1990) Repair of intrinsic DNA lesions, Mutation Res., 238, 305–311.
- Loeb, L.A. (1989) Endogenous carcinogenesis: molecular oncology into the twenty-first century — presidential address, Cancer Res., 49, 5489–5496.
- Loeb, L.A. (1991) Mutator phenotype may be required for multistage carcinogenesis, Cancer Res., 51, 3075–3079.
- Loeb, L.A., and K.C. Cheng (1990) Errors in DNA synthesis: a source of spontaneous mutations, Mutation Res., 238, 297–304.
- Loeb, L.A., and T.A. Kunkel (1982) Fidelity of DNA synthesis, Annu. Rev. Biochem., 51, 429–457.
- Loeb, L.A., and B.D. Preston (1986) Mutagenesis by apurinic/apyrimidinic sites, Annu. Rev. Genet., 20, 201–230.
- Luria, S.E., and M. Delbruck (1943) Mutations of bacteria from virus sensitivity to virus resistance, Genetics, 28, 491–511.
- Maron, D.M., and B.N. Ames (1983) Revised methods for the Salmonella mutagenicity test, Mutation Res., 113, 173–215.
- McGinty, L.D., and R.G. Fowler (1982) Visible light mutagenesis in *Escherichia coli*, Mutation Res., 95, 171–181.
- Meuth, M. (1990) The structure of mutation in mammalian cells, Biochim. Biophys. Acta, 1032, 1–17.
- Meyer, R.R., and P.S. Laine (1990) The single-stranded DNA-binding protein of *Escherichia coli*, Microbiol. Rev., 54, 342–380.
- Michaels, M.L., L. Pham, C. Cruz and J.H. Miller (1991)

- MutM, a protein that prevents G·C → T·A transversions, is formamidopyrimidine-DNA glycosylase, *Nucleic Acids Res.*, 19, 3629-3632.
- Modrich, P. (1987) DNA mismatch correction, *Annu. Rev. Biochem.*, 56, 435-466.
- Modrich, P. (1989) Methyl-directed DNA mismatch correction, *J. Biol. Chem.*, 264, 6597-6600.
- Mullaart, E., P.H.M. Lohman, F. Berends and J. Vijn (1990) DNA damage metabolism and aging, *Mutation Res.*, 237, 189-210.
- Naslund, M., D. Segerback and A. Kolman (1983) S-Adenosylmethionine, an endogenous alkylating agent, *Mutation Res.*, 119, 229-232.
- Nghiem, Y., M. Cabrera, C.G. Cupples and J.H. Miller (1988) The *mutY* gene: A mutator locus in *Escherichia coli* that generates G·C → T·A transversions, *Proc. Natl. Acad. Sci. (U.S.A.)*, 85, 2709-2713.
- Nishioka, H., and C.O. Doudney (1970) Different modes of loss of photoreversibility of ultraviolet light-induced true and suppressor mutations to tryptophan independence in an auxotrophic strain of *Escherichia coli*, *Mutation Res.*, 9, 349-358.
- O'Hara, S.M., and L.J. Marnett (1991) DNA sequence analysis of spontaneous and  $\beta$ -methoxy-acrolein-induced mutations in *Salmonella typhimurium hisD3052*, *Mutation Res.*, 247, 45-56.
- Phear, G., J. Nalbantoglu and M. Meuth (1987) Next-nucleotide effects in mutations driven by DNA precursor pool imbalances at the *aprt* locus of Chinese hamster ovary cells, *Proc. Natl. Acad. Sci. (U.S.A.)*, 84, 4450-4454.
- Piechocki, R., D. Kupper, A. Quinones and R. Langhammer (1986) Mutational specificity of a proof-reading defective *Escherichia coli dnaQ49* mutator, *Mol. Gen. Genet.*, 202, 162-168.
- Quah, S.-K., R.C. von Borstel and P.J. Hastings (1980) The origin of spontaneous mutation in *Saccharomyces cerevisiae*, *Genetics*, 96, 819-839.
- Quickenden, T.I., M.J. Comarmond and R.N. Tilbury (1985) Ultraweak bioluminescence spectra of stationary phase *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, *Photochem. Photobiol.*, 41, 611-615.
- Quinones, A., and R. Piechocki (1985) Isolation and characterization of *Escherichia coli* antimutators, A new strategy to study the nature and origin of spontaneous mutations, *Mol. Gen. Genet.*, 201, 315-322.
- Radman, M., and R. Wagner (1986) Mismatch repair in *Escherichia coli*, *Annu. Rev. Genet.*, 20, 523-538.
- Ramel, C. (1989) The nature of spontaneous mutations, *Mutation Res.*, 212, 33-42.
- Rebeck, G.W., and L. Samson (1991) Increased spontaneous mutation and alkylation sensitivity of *Escherichia coli* strains lacking the *ogt* O<sup>6</sup>-methylguanine DNA repair methyltransferase, *J. Bacteriol.*, 173, 2068-2076.
- Ripley, L.S. (1990) Frameshift mutation: determinants of specificity, *Annu. Rev. Genet.*, 24, 189-213.
- Rivas, E.I., A. Paladini Jr. and G. Cilento (1984) Photochemical-like destruction of tryptophan in serum albumins induced by enzyme-generated triplet species, *Photochem. Photobiol.*, 40, 565-568.
- Roberts, J.D., and T.A. Kunkel (1986) Mutational specificity of animal cell DNA polymerases, *Environ. Mutagen.*, 8, 769-789.
- Rudiger, H.W. (Ed.) (1990) Carcinogenic Risk by Endogenous Factors and Processes, *Mutation Res.*, 238, 173-329.
- Sankaranarayanan, K. (1991) Ionizing radiation and genetic risks, III. Nature of spontaneous and radiation-induced mutations in mammalian in vitro systems and mechanisms of induction of mutations by radiation, *Mutation Res.*, 258, 75-97.
- Sargentini, N.J., and K.C. Smith (1981) Much of spontaneous mutagenesis in *Escherichia coli* is due to error-prone DNA repair: implications for spontaneous carcinogenesis, *Carcinogenesis*, 2, 863-872.
- Sargentini, N.J., and K.C. Smith (1985) Spontaneous mutagenesis: the roles of DNA repair, replication, and recombination, *Mutation Res.*, 154, 1-27.
- Sargentini, N.J., and K.C. Smith (1986) Mutagenesis by normal metabolites in *Escherichia coli*: phenylalanine mutagenesis is dependent on error-prone DNA repair, *Mutation Res.*, 161, 113-118.
- Sargentini, N.J., and K.C. Smith (1989) Mutational spectrum analysis of *umuC*-independent and *umuC*-dependent  $\gamma$ -radiation mutagenesis in *Escherichia coli*, *Mutation Res.*, 211, 193-203.
- Sargentini, N.J., and K.C. Smith (1991) Involvement of RecB-mediated (but not RecF-mediated) repair of DNA double-strand breaks in the  $\gamma$ -radiation production of long deletions in *Escherichia coli*, *Mutation Res.*, 265, 83-101.
- Sargentini, N.J., W.P. Diver and K.C. Smith (1983) The effect of growth conditions on inducible, *recA*-dependent resistance to X rays in *Escherichia coli*, *Radiation Res.*, 93, 364-380.
- Savva, D. (1982) Spontaneous mutation rates in continuous cultures: the effect of some environmental factors, *Microbios*, 33, 81-92.
- Schaaper, R.M. (1988) Mechanisms of mutagenesis in the *Escherichia coli* mutator *mutD5*: Role of DNA mismatch repair, *Proc. Natl. Acad. Sci. (U.S.A.)*, 85, 8126-8130.
- Schaaper, R.M. (1989) *Escherichia coli* mutator *mutD5* is defective in the *mutHLS* pathway of DNA mismatch repair, *Genetics*, 121, 205-212.
- Schaaper, R.M., and R.L. Dunn (1991) Spontaneous mutation in the *Escherichia coli lacI* gene, *Genetics*, 129, 317-326.
- Schaaper, R.M., and L.A. Loeb (1981) Depurination causes mutations in SOS-induced cells, *Proc. Natl. Acad. Sci. (U.S.A.)*, 78, 1773-1777.
- Schaaper, R.M., B.N. Danforth and B.W. Glickman (1986) Mechanisms of spontaneous mutagenesis: an analysis of the spectrum of spontaneous mutation in the *Escherichia coli lacI* gene, *J. Mol. Biol.*, 189, 273-284.
- Schaaper, R.M., R.L. Dunn and B.W. Glickman (1987) Mechanisms of ultraviolet-induced mutation, Mutational spectra in the *Escherichia coli lacI* gene for a wild-type and an excision-repair-deficient strain, *J. Mol. Biol.*, 198, 187-202.
- Shankel, D.M., P.E. Hartman, T. Kada and A. Hollaender (Eds.) (1986) Antimutagenesis and Anticarcinogenesis Mechanisms, (Basic Life Sci., Vol. 39), Plenum, New York.
- Sharma, R.C., and K.C. Smith (1985) A mechanism for rich-

- medium inhibition of the repair of daughter-strand gaps in the deoxyribonucleic acid of UV-irradiated *Escherichia coli* K12 *uvrA*, *Mutation Res.*, 146, 177–183.
- Sharma, R.C., T.R. Barfknecht and K.C. Smith (1982) Postreplication repair in *uvrA* and *uvrB* strains of *Escherichia coli* K-12 is inhibited by rich growth medium, *Photochem. Photobiol.*, 36, 307–311.
- Shibutani, S., M. Takeshita and A.P. Grollman (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG, *Nature (London)*, 349, 431–434.
- Simic, M.G., D.S. Bergtold and L.R. Karam (1989) Generation of oxy radicals in biosystems, *Mutation Res.*, 214, 3–12.
- Smith, K.C., and N.J. Sargentini (1985) Metabolically-produced 'UV-like' DNA damage and its role in spontaneous mutagenesis, *Photochem. Photobiol.*, 42, 801–803.
- Smith, K.C., and N.J. Sargentini (1986) Mechanisms of spontaneous mutagenesis: implications for spontaneous carcinogenesis, in: F.J. Burns, A.C. Upton and G. Silini (Eds.), *Radiation Carcinogenesis and DNA Alterations*, Plenum, New York, pp. 359–371.
- Smith, K.C., and R.C. Sharma (1987) A model for the *recA*-dependent repair of excision gaps in UV-irradiated *Escherichia coli*, *Mutation Res.*, 183, 1–9.
- Smith, K.C., and T.V. Wang (1989) *recA*-Dependent DNA repair processes, *BioEssays*, 10, 12–16.
- Smith, M.W., and F.C. Neidhardt (1983a) Proteins induced by anaerobiosis in *Escherichia coli*, *J. Bacteriol.*, 154, 336–343.
- Smith, M.W., and F.C. Neidhardt (1983b) Proteins induced by aerobiosis in *Escherichia coli*, *J. Bacteriol.*, 154, 344–350.
- Sohal, R.S., and R.G. Allen (1990) Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis, *Exptl. Gerontol.*, 25, 499–522.
- Sowers, L.C., B. Ramsay Shaw, M.L. Veigl and W.D. Sedwick (1987) DNA base modification: ionized base pairs and mutagenesis, *Mutation Res.*, 177, 201–218.
- Stewart, F.M., D.M. Gordon and B.R. Levin (1990) Fluctuation analysis: the probability distribution of the number of mutants under different conditions, *Genetics*, 124, 175–185.
- Storz, G., M.F. Christman, H. Sies and B.N. Ames (1987) Spontaneous mutagenesis and oxidative damage to DNA in *Salmonella typhimurium*, *Proc. Natl. Acad. Sci. (U.S.A.)*, 84, 8917–8921.
- Strauss, B.S. (1991) The 'A rule' of mutagen specificity: a consequence of DNA polymerase bypass of non-instructional lesions? *BioEssays*, 13, 79–84.
- Tang, M.S., T.V. Wang and M.H. Patrick (1979) DNA turnover in buffer-held *Escherichia coli* and its effect on repair of UV damage, *Photochem. Photobiol.*, 29, 511–520.
- Tchou, J., H. Kasai, S. Shibutani, M.-H. Chung, J. Laval, A.P. Grollman and S. Nishimura (1991) 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity, *Proc. Natl. Acad. Sci. (U.S.A.)*, 88, 4690–4694.
- Terleth, C., P. van de Putte and J. Brouwer (1991) New insights in DNA repair: preferential repair of transcriptionally active DNA, *Mutagenesis*, 6, 103–111.
- Topal, M.D., and J.R. Fresco (1976) Complementary base pairing and the origin of substitution mutations, *Nature (London)*, 263, 285–289.
- Totter, J.R. (1980) Spontaneous cancer and its possible relationship to oxygen metabolism, *Proc. Natl. Acad. Sci. (U.S.A.)*, 77, 1763–1767.
- Trosko, J.E., and C.-C. Chang (1976) Role of DNA repair in mutation and cancer production, in: K.C. Smith (Ed.), *Aging, Carcinogenesis, and Radiation Biology (The role of nucleic acid addition reactions)*, Plenum, New York, pp. 399–442.
- van der Schueren, E., D.A. Youngs and K.C. Smith (1974) Sensitization of ultraviolet-irradiated *Escherichia coli* K-12 by different agars: inhibition of a *rec* and *exr* gene-dependent branch of the *uvr* gene-dependent excision-repair process, *Photochem. Photobiol.*, 20, 9–13.
- von Borstel, R.C. (1969) On the origin of spontaneous mutations, *Jpn. J. Genet.*, 44 (Suppl. 1), 102–105.
- Wang, T.V., and K.C. Smith (1986) Inviability of *dam recA* and *dam recB* cells of *Escherichia coli* is correlated with their inability to repair DNA double-strand breaks produced by mismatch repair, *J. Bacteriol.*, 165, 1023–1025.
- Wang, T.V., and K.C. Smith (1989) The roles of RecBCD, Ssb and RecA proteins in the formation of heteroduplexes from linear-duplex DNA in vitro, *Mol. Gen. Genet.*, 216, 315–320.
- Warren, S.T., R.A. Schultz, C.-C. Chang, M.H. Wade and J.E. Trosko (1981) Elevated spontaneous mutation rate in Bloom syndrome fibroblasts, *Proc. Natl. Acad. Sci. (U.S.A.)*, 78, 3133–3137.
- Washburn, B.K., and S.R. Kushner (1991) Construction and analysis of deletions in the structural gene (*uvrD*) for DNA helicase II of *Escherichia coli*, *J. Bacteriol.*, 178, 2569–2575.
- Watson, J.D., and F.H.C. Crick (1953) The structure of DNA, *Cold Spring Harbor Symp. Quant. Biol.*, 18, 123–131.
- Webb, R.B., and J.R. Lorenz (1972) Toxicity of irradiated medium for repair-deficient strains of *Escherichia coli*, *J. Bacteriol.*, 112, 649–652.
- Webb, R.B., and M.M. Malina (1970) Mutagenic effects of near ultraviolet and visible radiant energy on continuous cultures of *Escherichia coli*, *Photochem. Photobiol.*, 12, 457–468.
- Weston-Hafer, K., and D.E. Berg (1991) Deletions in plasmid pBR322: replication slippage involving leading and lagging strands, *Genetics*, 127, 649–655.
- Wintersberger, U. (1991) On the origins of genetic variants, *FEBS Lett.* 285, 160–164.
- Witkin, E.M. (1967) Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli* B differing in sensitivity to ultraviolet light, *Brookhaven Symp. Biol.*, 20, 17–55.
- Witkin, E.M. (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*, *Bacteriol. Rev.*, 40, 869–907.