Mutagenesis

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INTRODUCTION

Mutagenesis is better understood in *Escherichia coli* than in any other organism for several reasons: the biology of *E. coli* is known more completely than that of any other organism; mutagenesis is readily observed because the cell is genetically haploid, so a mutation in only one copy of a gene is needed for phenotypic expresssion; the SOS response in *E. coli* (chapter 89, this volume) has greatly aided dissection of mutagenic processes; the use of coliphages has made possible studies of mutagenesis in the simpler viral genomes, with the possibility of treating DNA and cell separately; and it is easy to manipulate the large numbers of *E. coli* cells needed for analyzing the relatively rare process of mutagenesis. The rapid and extensive development of molecular biology has made possible the extrapolation of insights from this prokaryote to other organisms, including humans.

The purpose of this chapter is to provide a coherent and readily understood picture of what is known about mutagenesis of *E. coli.* The literature is enormous, and no attempt at completeness of coverage has been made. Those aspects that are generally accepted are presented as simply as possible, with minimal reference to the original literature. This approach can make some areas appear better understood than is in fact the case, but this disadvantage is outweighed by the ease with which the main outlines of mutational processes can be presented, without requiring the reader to plow through the mass of results that went into determining each part of the picture. Matters that are not well understood, or about which there is controversy, are documented much more extensively, with detailed references to the original literature.

There is an extensive review of mutagenesis by G. Walker in the recent text *DNA Repair and Mutagenesis* (64). Other reviews of *E. coli* mutagenesis include those of Eisenstadt (57), Miller (117, 119), Sedgwick (160), and Bridges (20). More specialized reviews include those on frameshift mutagenesis (144), spontaneous mutation (47), replication fidelity (55), DNA replication and mutagenesis (106), the SOS response (182), T4 bacteriophage mutagenesis (49, 50), and DNA damage in untreated cells (105). Finally, two “golden oldies,” Drake’s 1970 book (45) and Witkin’s 1976 review (188), are still worth perusal for their summing up of particular aspects of mutagenesis and for their insights.

Mutations are usually classified as base substitutions or base changes; deletions, or the loss of any number of base pairs; insertions, or the addition of any number of base pairs; inversions, in which a piece of the linear DNA molecule is cut out and reinserted in reverse order; duplications, a form of insertion in which the added bases are the same as a sequence already in the genome, usually that immediately adjacent to the insert; and complex mutations, which may include any combination of these mutations in a single genome. Base substitutions are sometimes further classified as transitions, a purine changed to a purine or a pyrimidine changed to a pyrimidine, and transversions, a purine changed to a pyrimidine or vice versa. It is convenient (and conventional) to use the term *frameshift* for the loss or addition of one or two base pairs; those situations in which the term frameshift is used to include larger deletions or insertions that change the reading frame during the process of translation are generally self-evident.

HISTORICAL ASPECTS OF *E. COLI* MUTAGENESIS

Until the 1930s, bacteriologists were primarily interested in the properties of a bacterial culture as a whole (176). The extraordinary changes that take place in cultures grown under various conditions, from emergence of colonies utilizing lactose in a culture unable to grow on that sugar (enzyme induction) to smooth colonies appearing in a culture that made only rough colonies (mutation), were ascribed to "bacterial variation." As late as 1945, Dubos could write in a standard text (52): "Except in a few suggestive cases, . . . there is no evidence that bacterial variation behaves according to Mendelian laws."

A shift in focus to properties attributable to the individual cells became significant in the 1930s, in large part because of studies of radiation effects on bacteria by O. Rahn, F. L. Gates, R. W. G. Wyckoff, D. E. Lea, and J. W. Gowen, among others (176). In 1943, the Luria-Delbrück experiment (108) measured the appearance of mutants resistant to coliphage T1 in growing cultures of *E. coli.* The observed distributions were inconsistent with mutations induced by the presence of the phage (that is, unlike enzyme induction) but consistent with random appearance during cell replication, encouraging workers to regard bacterial genetics as similar to the well-established genetics of *Drosophila melanogaster* and maize.

Conclusive evidence that DNA is the carrier of genetic information was provided by the demonstration that a heritable characteristic could be transferred from one type of *Pneumococcus* cell to another by transfer of DNA and only DNA (7). This epochal finding was brought to the attention of many by the Hershey-Chase experiment, showing that propagation of a coliphage requires injection of the ^{32}P-labeled phage DNA, but not the 35S-labeled protein, into the *E. coli* host cell (72). Genetic mapping in coliphage T4 with resolution on the order of a single base by Benzer (14) made possible the demonstration of the triplet nature of the genetic code (37). Certain mutations were dubbed "nonsense" because they made triplet codons that did not code for an amino acid (15), and these codons were then shown to be chain terminators (TAG, TAA, and TGA) whose effects were suppressed by mutant tRNAs that inserted an amino acid at these codons (175).

With these fundamental results, an understanding of mutagenesis on a molecular level became feasible.

DETERMINATION OF MUTAGENESIS IN *E. COLI*

There are two general types of mutation. A forward mutation is a change in the normal or wild-type gene sequence, most commonly detected as a change (usually decrease) in gene product activity. In addition to changes that affect a protein, forward mutations can change the genes for tRNA or rRNA and also the sequences that control the initiation and termination of RNA transcription or DNA replication as well as ribosomal binding. A revertant is a cell with a mutated gene or sequence, as described above, that has regained a phenotype resembling wild type because of a second mutation.

Although *E. coli* is genetically haploid, most cells contain two or more copies of the genome. A forward mutation that inactivates a gene cannot readily be identified until the mutated gene has segregated by cell division and the level of unmutated gene product has decreased sufficiently by inactivation and dilution by cell division to allow phenotypic expression. Offsetting these difficulties, studies of forward mutagenesis have the advantage of including all kinds of mutations.

Reversions are frequently easier to detect and more readily quantitated. On the other hand, only specific mutations that revert a mutated gene back to active form are detected.

Reversion Mutations

Many investigations of mutagenesis in *E. coli,* especially between 1950 and 1980, used strains with a mutation that makes growth dependent on an additive in the medium (auxotrophs) and studied revertants that can grow without the supplement (prototrophs). Such revertants are readily identified, and phenotypic expression is fast, with no need for gene segregation or loss of gene product activity.

In the usual reversion assay, mutagenized cells are spread on plates with agar-containing medium on which prototrophic revertants, but not the auxotroph, will grow, enriched with a small amount of the additive (semienriched medium or SEM plates) to allow sufficient DNA replication to "fix" the mutation. (On

occasion, fixation occurs without additions because traces of essential nutrients in the cells, agar, or medium allow some replication.) If quantitative mutation frequencies are wanted, it is essential to determine the number of viable cells under the same conditions used to score mutants, because the probabilities of survival and of mutation are greatly influenced by details of the course of events as the cell recovers from genotoxic insult. Since reversion of a particular mutation is a rather rare event, it is usual to put 10^6 to 10^9 mutagenized cells on one 100-mm-diameter petri plate, and this can give rise to a cell density artifact (188): the fraction of cells that divide often enough to form microcolonies can depend on the number of cells plated, and nutrients leaking from dead cells unable to divide can increase the number of divisions for surviving cells. The difficulty is particularly severe for bacterial cells such as *E. coli* K-12 that, when treated with genotoxic agents, form filaments that are very large compared with the size of a normal cell and rapidly deplete available nutrients.

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Characterization of Revertant Mutations. A cell that is auxotrophic because of a base change mutation can revert by restoration of the original base sequence or by some other mutation: one that inserts a tolerated amino acid at the mutated site, or a second-site mutation that changes another amino acid in the protein, or even in another protein that interacts with the mutated molecule, to give increased product activity. For an auxotroph with a nonsense mutation, a base change in a tRNA gene can make a suppressor tRNA (see below). Frameshifts may revert to give the original base sequence, or reversion may be effected by nearby frameshifts that restore the original translational reading frame without introducing stop codons in the coding sequence or unacceptable changes in amino acids.

Revertants that have reverted because of different mutations can be distinguished sometimes by phenotype (such as colony morphology) and sometimes by appearance or disappearance of a suppressible stop codon or of a restriction enzyme site in the DNA. Sequencing of the DNA is always possible but a lot of work. In some cases, the most effective method is blotting: a labeled oligonucleotide (typically 12 to 20 bases long), with the exact sequence of an expected mutation and annealed to revertant DNA, is significantly more difficult to elute at high washing temperatures than oligonucleotides differing by only a single base (122).

A strain can be annoying to work with if revertants form colonies of variable size and appearance that slowly arise during several days of incubation (i.e., are "leaky"). Thus, it is common to search for strains with uniform and nonleaky revertants. In the early 1950s, Evelyn Witkin isolated and characterized two *E. coli* B strains, WP2(*trp*) and WU36(*tyr*), that have a low spontaneous reversion rate to prototrophy but are induced to revert by typical mutagens at a high rate, with revertants that are easy to assay and form colonies of uniform size and appearance (186). Revertants of these strains are typically 90% suppressor mutations and 10% base changes in the mutated codon. Various derivatives of these strains have been widely used in many experiments that have contributed much to present knowledge, but the implied assumption that the observed mutagenesis is representative has on occasion turned out to be incorrect.

Nonsense Suppressors. Some base substitutions change the codon for an amino acid to a nonsense codon that is a signal to stop translation, and the mutant polypeptide is truncated. Genes with these extra stop codons—TAG (amber), TAA (ochre), or TGA (UGA or opal)—can revert either by a base change in the stop codon or by the formation of a suppressor mutation in a tRNA gene that alters the tRNA anticodon to the complement of the stop codon and inserts the related amino acid in the gene product. Note that various amino acids are inserted by different suppressors; even with the restriction to single base changes in the tRNA gene, an amber (TAG) suppressor can insert any one of seven amino acids: lysine (AAG), glutamine (CAG), valine (GAG), serine (TCG), tryptophan (TGG), leucine (TTG), or tyrosine (TAC or TAT). The amino acids inserted by specific tRNA suppressors are listed in section 20 of reference 119. In some cases, insertion of any amino acid will give a protein of sufficient activity to

score as a revertant, but in other cases, only tRNAs inserting the original amino acid will suppress the mutation.

Amber suppressors are the most common, ochre suppressors are less common, and UGA (or opal) suppressors are rarely found because TGA is the most common terminator codon and TGA suppressors can make a cell nonviable.

Ames Test. In a comprehensive application of reversion to detect mutagenesis, tester strains of *Salmonella typhimurium* (official designation, *Salmonella enterica* serovar Typhimurium) with various mutations in the *his* gene have been selected by Ames and coworkers (4, 114) for the ability to detect different base substitutions and frameshifts. These strains have various DNA repair deficiencies to minimize loss of mutagenic lesions from the DNA and thus increase sensitivity to mutagenic effects; they have genetic defects that make cell membranes more permeable to chemical agents; many of the strains incorporate plasmid pKM101, which greatly increases mutability by agents making DNA lesions which block polymerases. Metabolic processing in mammalian cells that converts many chemicals (such as benzpyrene) to mutagenic form can be mimicked by adding mammalian microsomal fractions to the assay plates (114). Simplicity and the broad understanding of bacterial mutagenesis have made the Ames test the most widely used procedure for detection of mutagens. *Important:* As the Ames test is usually used, results are generally not quantitative except when mutagenic treatment has negligible effects on colony-forming ability.

Six *E. coli* Lac^- strains with mutations in the *lacZ* gene have been constructed, each reverting to Lac+ only by one of the six possible base changes (39). Since mutagenesis typically depends strongly on adjoining bases, obtaining data for a given base change in only one sequence context is a significant limitation. A second set revert only by specific frameshifts in runs of A's (T's in the complementary strand) or G's (or C's) or in an alternating -G-C- sequence (38).

Forward Mutations

Forward mutations in genes for which there is an assay are readily detected: mutagenize a culture, grow it under nonselective conditions for phenotypic expression, and assay. If there is a much larger fraction of mutants in the mutagenized culture than in an untreated culture processed in a similar way, it is customarily assumed that most of the mutations in the treated culture arose from the mutagen. However, the number of mutants per colony former in the culture after growth does not give a quantitative measure of the number of mutational events because individual treated cells can, and frequently do, grow and divide at greatly different rates.

If treated cells are spread on agar before division, quantitative mutation rates are obtained if all survivors grow and a suitable assay detects mutant cells. A widely used assay utilizes the well-studied *lac* operon (12, 119) and depends on the ability of β-galactosidase, product of the *lacZ* gene, to cleave the indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) to give a blue color. Wild-type *E. coli* cells have an active LacI repressor that turns off the *lac* operon, including *lacZ,* and therefore form white colonies. Mutagenized *E. coli* cells on agar with X-Gal form mostly white $lacI^+$ colonies, with a few all-blue colonies from cells that were *lacI* (defective for the LacI repressor) before treatment, and colonies with white and blue sectors for those in which the *lacI* gene was mutagenized (119), the sectors corresponding to segregation of $lacI^+$ and *lacI* genes.

A related assay may be used to detect mutations in a 290+-bp fragment of the *lacZ* gene, typically inserted in M13 phage or on single- or double-stranded DNA that will replicate in cells after transfection. The LacZ(α) polypeptide from this fragment complements the product of a suitably modified *lacZ* chromosomal gene to produce active β-galactosidase and form a blue product; mutations in the α-complementing fragment inactivate the enzyme and give a white colony (115, 119).

Coliphages are very useful in mutagenesis studies. The molecular biology of bacteriophage T4 has been extensively explored (85, 111); the *r*II locus is a favorite target for mutagenesis (49, 50) and was used in much of the early work on fine-structure genetic mapping and on the genetic code (see above). Since T4 does not use host cell replication or repair enzymes, much recent work has shifted to the well-known phage λ,

which depends on both (71). The clear-plaque phenotype produced by a mutation in the *c*I repressor gene of λ is readily distinguished from the wild-type turbid form, and quantitative determinations are possible for mutagenesis of the gene on phage DNA integrated into the host genome as a prophage, for phage DNA as a plasmid inside the cell, as free phage (thus avoiding action of the mutagenic agent on the host cell), and as free DNA in solution that can be repackaged into phage for assay (78).

Base Sequence Changes in Mutagenesis

Information on base sequence changes was obtained indirectly as early as 1966, by sequencing the mutant protein and deducing the base changes through a knowledge of the genetic code (194).

Most frequently, changes in DNA base sequence are determined by sequencing, often by amplifying the gene DNA by PCR (129, 130) and then sequencing either by chain termination methods (150) or by base-specific cleavage (112). While any assayable gene can be used, most determinations of sequence changes in mutagenesis use certain genes that have been repeatedly sequenced: the procedures are well worked out in the literature, special cell strains and phage and/or plasmids are usually available, and there are comparative data for other mutagens. A computer database of sequence changes in various *E. coli* genes contained over 13,000 mutations (December 1993) and is available on Internet (77).

The *lacI* gene for the Lac repressor is the most widely used for sequencing mutations, partly because it was the first for which extensive data were available (35), partly because the lactose operon is well understood (12, 119), and partly because there is a quick method for determining certain mutational sequence changes (see below). The LacZ(α)-complementing factor is available on single-stranded M13 viruses, and sequencing can be done easily, without PCR, from amplified viral DNA and with widely available primers (115). Phage λ has been extensively studied (71), and its *c*I repressor gene can be readily sequenced after being mutagenized on a prophage in the host *E. coli* genome, on a plasmid in host cells or on free phage, or on free DNA in solution (78). The *gpt* gene for the *E. coli* xanthine guanine phosphoribosyltransferase enzyme is readily expressed and assayed in both bacterial and mammalian cells (128) and is particularly useful for studies comparing bacterial and mammalian mutagenesis.

An ingenious method for rapidly determining sequence changes at nonsense mutations in a *lacI* gene on an F′ episome was developed by Jeffrey Miller and his associates before DNA sequencing became routine (34, 35). Each amber and ochre mutation is precisely located by crosses with appropriate deletion mutants (120); the base change is deduced from the knowledge of the particular amber or ochre mutation and the gene sequence. The advantage is that much larger numbers of mutations can be characterized than is presently feasible with sequencing; the disadvantage is that only certain mutations can be characterized: all single-base changes except A:T to G:C, but only in certain sequence contexts, and a very few tandem base changes.

MUTAGENESIS BY EXOGENOUS AGENTS

Dependence on Mutagenic Exposure

Genotoxic agents kill and mutagenize cells by damaging DNA. In many cases, survival of bacteria is roughly exponential with dose, and mutant fraction increases more or less linearly with dose. That is, an increment of dose dD changes the number n of viable cells by

$$dn = -\sigma\, n\, dD \qquad (1)$$

where σ specifies the effect of the agent on cell survival. The same increment of dose changes the number of mutants m

$$dm = \eta\, n\, dD - \sigma\, m\, dD \qquad (2)$$

where η specifies the effectiveness of the mutagen in inducing new mutations and the second term is the number of mutants in the culture killed by the dose increment. Equation 1 is readily integrated.

$$n = N \exp(-\sigma D) \qquad (3)$$

Substituting equation 3 in equation 2 gives a linear first-order differential equation with a well-known solution that can be written

$$m/n = \eta D + M/N \qquad (4)$$

where N and M are the numbers of viable and mutant cells, respectively, in the original culture. In words, assuming one-hit effects, the same lesion can either mutate or kill a cell but not both; equation 2 incorporates the assumption that mutated and unmutated cells have the same probability of surviving.

The numbers of viable bacteria and mutants sometimes show more complicated dependence on dose; for example, mutation sometimes depends on the square of dose (66, 188).

The SOS Response

The role of the SOS response in *E. coli* mutagenesis can be most simply described experimentally in terms of mutagenesis of coliphage λ, whose DNA is repaired and replicated by essentially the same enzymes as the cell chromosome. Phage λ, irradiated with UV light and adsorbed to *E. coli* host cells, produces mutated phage only when the host cells are also irradiated (184). Irradiating the host cell induces the SOS response needed for UV mutagenesis, and the event that triggers the SOS response is inhibition of DNA replication, principally by replication complexes blocked at lesions in the template (chapter 89).

Mutagenesis of a gene on the cell chromosome occurs by the same process: UV both induces the SOS response, as described above, and forms photoproducts in the mutated gene, equivalent to irradiating the phage. No UV-induced mutations are observed in *rec*A cells (124) that lack the SOS response or in *umu*C or *umu*D cells (87, 173) that do not make the SOS-dependent gene products essential for UV mutagenesis.

Wild-type *S. typhimurium* cells are not very mutable by UV but become so with the introduction of plasmid R46 (126). The same effect is produced by plasmid pKM101, derived from R46, which codes for two genes, *mucA* and *mucB,* that are homologous to *umuD* and *umuC,* respectively, and have similar functions (137; chapter 89).

Polymerase at a Lesion in Template DNA

The principal cause of mutation induced by an agent that damages DNA is a polymerase encountering a lesion in a template (Fig. 1). First, the polymerase extends the growing 3′ end by addition of a nucleotide opposite the lesion. Next, there is competition between removal of that nucleotide by the 3′→5′ proofreading exonuclease of the holoenzyme and extension by addition of still another nucleotide. If addition of either nucleotide is slow, progression of the polymerase is blocked. In most cases, the rate-limiting process is the second step, addition to the nucleotide opposite the lesion (106, 167).

Unassisted Translesion Synthesis. Some agents mutagenize in the absence of an SOS response; the polymerase can synthesize past the lesions, presumably because the lesions make good enough base pairs with normal bases to allow chain extension. The base analog bromouracil is similar stereochemically to T, with a bromine atom replacing the methyl group, and forms a good base pair with A; it can also pair with G at low frequency, and when this pair eludes repair, a T:A-to-C:G mutation is formed. O^6-Methylguanine (Fig. 2), formed by the action on G of methylating agents such as *N*-methyl-*N*-nitrosourea or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, pairs readily with either C or T (102, 191) with efficiencies that depend on sequence; the latter pairing mutates G:C to A:T.

SOS-Assisted Translesion Synthesis. Unassisted translesion synthesis is so slow as to be essentially unobservable, in most cases, for lesions such as the most common UV photoproducts in double-stranded DNA: *cis-syn* dipyrimidine cyclobutane dimers and pyrimidine (6–4) pyrimidinones (Fig. 2). Polymerase III (Pol III) holoenzyme can replicate past such lesions only with the aid of a complex of SOS proteins UmuC and UmuD′ (UmuD′ is the processed form of the UmuD protein; see chapter 89) and RecA (65a, 142); no other SOS operon need be induced (172). Even though other polymerases can

replicate the genome in the absence of Pol III α polymerase (the product of the *dna*E gene), there is evidence that SOS-assisted translesion synthesis probably occurs only with the Pol III holoenzyme with the α component (21, 25, 127). Many DNA lesions, including abasic sites (missing bases in the DNA), thymine glycols (Fig. 2), and bulky groups (such as aflatoxin) attached to a base, also require SOS assistance for translesion synthesis. The process is discussed in detail, with extensive references to the literature, in reference 64.

Translesion synthesis is mutagenic when wrong bases, or wrong numbers of bases, are inserted in the newly synthesized strand, and the SOS-assisted process accounts for nearly all "inducible error-prone repair" cited so frequently in the older literature; there is probably also a small component arising from SOS-related recombination.

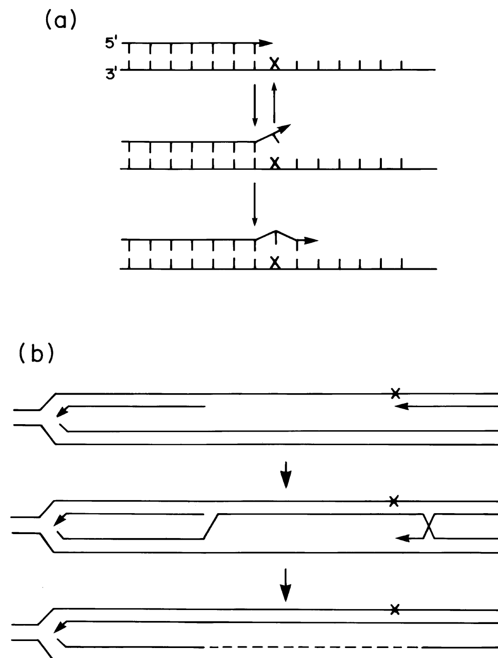

FIGURE 1 Bypass of a lesion (X) in template DNA blocking a replication complex (arrowhead). (a) Bypass by translesion synthesis, with and without SOS assistance. (b) Bypass by postreplication repair. Replication is reinitiated downstream (first figure); the gap is filled in by recombination with the sister strand (second figure), followed by DNA synthesis (dashed line) and resolution of the Holliday-type junction (see chapter 121).

Some agents make two kinds of lesions in DNA: those that are readily synthesized past and also those for which translesion synthesis is significant only with SOS assistance. Ionizing radiation, for example, induces mutations in phage λ assayed in uninduced cells or in *umuC* or *recA* hosts without SOS functions, showing the presence of readily bypassed lesions; a 7- to 10-fold increase in mutants per survivor for phage assayed in SOS-induced host cells reveals additional lesions that are mutagenic with SOS-assisted bypass (19, 180).

Postreplication Repair. In most cases, a blocked polymerase reinitiates replication at a site thousands of bases downstream (147); the gap between the 3′ end of the newly formed strand at the lesion and the reinitiation site is filled by a process involving recombination with the sister duplex (Fig. 1b) (148; chapter 121). The process enables the error-free duplication of the genome but does not eliminate lesions from the DNA. Postreplication repair is essentially normal in *umuDC* cells with little or no UV-induced mutagenesis (86) and so must be essentially error free.

Competition between Modes of Bypass. Each of the three processes—unassisted translesion synthesis, SOS-assisted translesion synthesis, and postreplication repair—can occur at any particular lesion. The relative contribution of each determines both the frequency and the kind of mutation and depends on the lesion, DNA sequence, and state of the cell. There is competition between SOS-assisted translesion synthesis and postreplication repair at UV photoproducts (normally not bypassed without SOS assistance): in *umuDC* cells with no translesion synthesis (and without mutation), a small decrease in survival (87) is attributable to loss of bypass.

Competition between unassisted and SOS-assisted translesion synthesis occurs in single-strand DNA at a UV-induced *trans-syn* T↔T cyclobutane dimer (Fig. 2), a photoproduct found in single-stranded but not double-stranded DNA: without SOS, it causes a low frequency of single T deletions; SOS-assisted translesion synthesis causes a much higher frequency of mutations, mostly single base changes with a few –T frameshifts (9). Possibly, *lacI* mutations induced by UV in *umuC* cells that were allowed to multiply many times (28) come from a low frequency of translesion synthesis past photoproducts that have been repeatedly bypassed by postreplication repair but not eliminated from the DNA in the excision-deficient cells.

An attractive hypothesis is that hindering error-free postreplication repair might allow more time for translesion synthesis and therefore mutation; postreplication repair of one lesion might be reduced by a second lesion on the complementary strand and within a distance comparable to the size of the gap formed (Fig. 1b) (18, 159). This might account for the quadratic dependence on dose of mutations in cells in which the SOS response is fully induced—e.g., for UV (188) or gamma rays (66).

It is helpful to realize that for most DNA lesions, only a very small fraction cause either cell killing or mutation. For example, a UV fluence of 1 J/m^2 to *E. coli* unable to excise photoproducts forms about 60 photoproducts in the genome (147) and kills about half the cells, so only 1 photoproduct in 100 is lethal. *E. coli* cells lacking both excision and postreplication repair are killed by an estimated one to two UV photoproducts per genome (74), so the ability of excision-defective cells to survive with tens of photoproducts in the DNA is ascribed to postreplication repair. A UV fluence of 1 J/m^2 induces 0.012 photoproducts in a typical 1,000-bp gene and 1 × 10–5 to 10 × 10–5 mutations per survivor, so only 1 photoproduct in 1,000 causes a mutation.

Mutagenesis by a Defined Lesion at a Specific Site

Useful data on the frequency and kinds of mutations induced by a particular lesion can be obtained by incorporating the lesion at a specific site in a gene on a DNA construction that can replicate after transfection into cells (169). Mutation frequency is the ratio of cells with mutant genes to cells with plasmids (identified by a marker gene, e.g., for drug resistance). The sequence changes in newly replicated DNA can be determined readily (Table 1), in some cases by changes in a suppressible mutation or restriction enzyme site. Modified bases are frequently incorporated in single-stranded circular DNA, which eliminates (or greatly reduces) effects of DNA repair. The use of single-stranded DNA also avoids a serious complication with double-stranded DNA: selective replication of the strand without the lesion. However, extrapolation from data for single-stranded DNA to the effects of a modified base in double-stranded DNA must be done with care. Also, the sequence surrounding the lesion has important effects (see, for example, the two abasic sites listed in Table 1).

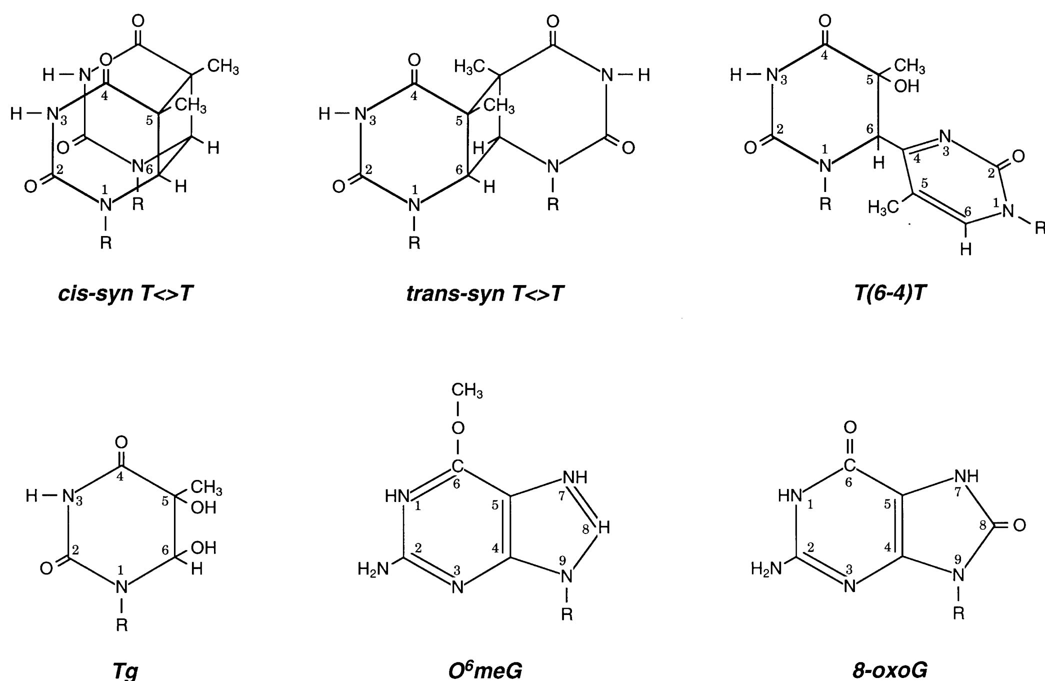

FIGURE 2 Typical mutagenic lesions in DNA. R is a sugar in the phosphate-deoxyribose backbone. T<>T, cyclobutane thymine dimer, *cis-syn* and *trans-syn* forms; T(6-4)T, the T-T pyrimidine-pyrimidinone (6-4) adduct; Tg, thymine glycol (there are four stereoisomers); O^6 meG, O^6-methylguanine; 8-oxoG, 8-oxo-7,8-dihydroguanine.

Table 1 confirms an earlier deduction (116) that lesions determine the kinds of mutations. The mutations are nearly always at the site of a lesion, only rarely at an adjoining base pair.

REPAIR AND MUTAGENESIS

Removal of Mutagenic Lesions from DNA

Removal of lesions from DNA by repair increases the number of surviving cells n and decreases the number of mutants m induced by a dose D of mutagen, as may be seen by comparing, for example, the response to UV of *E. coli* with and without nucleotide excision repair that removes photoproducts from the cell genome. Different lesions may be removed at different rates, and effects on cell survival and mutagenesis can be estimated, in cases for which equations 3 and 4 are valid, by considering both σ and η to be the sum of terms

$$\sigma = \sigma_1 + \sigma_2 + \ldots \quad \eta = \eta_1 + \eta_2 + \ldots \qquad (5)$$

A decrease in the ith product from its initial value to some smaller value reduces the corresponding σ_i and η_i accordingly.

Also, rate of removal usually differs from one site in the DNA to another. Consider sites a and b, at which there are p_a and p_b lesions per site. If fractions dp_a/p_a and dp_b/p_b are removed during some time interval, and if lesions at site b are removed at a rate k more rapidly than at a,

TABLE 1 Mutations induced by a lesion at a defined site in circular single-strand DNA transfected into *E. coli*

Lesion	SOS in cell	Translesion frequency	Mutational specificity	Reference(s)
Cyclobutane dimer				
cis-syn T ↔ T	–	0.005		
		0.0186	Of 2,608 mutations sequenced: TT → TT, 2,443; TT → TA, 130; TT → TC, 28; TT → CT, 6	10, 98
trans-syn T ↔ T	–	0.139	Of 389 mutations sequenced: TT → TT, 373; –T, 16	9
		0.285	Of 605 mutations sequenced: TT → TT, 536; –T, 33; TT → AT, 16; TT → CT, 4; TT → TC, 1; +frameshift, 9; –frameshift, 4; adjacent base changes, 2	9
Pyrimidine-pyrimidinone (6-4)				
T(6-4)T	–	0.019		
		0.22	Of 185 mutations sequenced: TT → TT, 16; TT → TC, 158; TT → CT, 2; TT → GT, 2; TT → TG, 1; TT → AC, 3; TT → CC, 2; –T, 1	100
Abasic site (X)				
-G-X-T-G-	–	0.006		
		0.069	Of 104 mutations sequenced: X → T, 52; X → A, 24; X → C, 19; X → G, 1; XT → AA, 1; –1 frameshift, 7	99
-G-T-X-G-	–	0.003		
		0.05	Of 100 mutations sequenced: X → T, 77; X → A, 4; X → C, 14; X → G, 1; –1 frameshift, 4	99
O^6-Methylguanine	–	High	G → A, 0.4% In repair-inhibited cells, G → A, ~20%	107
8-OxoG	–	High	G → T, 0.7%	192
Tg[a] sequence 5′-C-Tg-A-3′	–	High[b]	T → C, 0.2–0.4%	11
		High[b]	T → C, 0.2–0.4%	11

[a]Tg, thymidine glycol.
[b]In uninduced *E. coli,* Tg blocks polymerases very effectively exept at 5′C-Tg-Pur sequences (58), as in this example. In other sequence contexts in SOS-induced *E. coli,* the chance that replication forks bypass Tg rises to 60 to 70%, with the indicated fraction of transitions induced (58).

$$dp_b/p_b = k\ dp_a/p_a \qquad (6)$$

and by integration,

$$p_b/P_b = (p_a/P_a)^k \qquad (7)$$

where P_a and $\boldsymbol{P_b}$ are the initial numbers of lesions at the sites. Assume, for example, that site a is an average site at which 99% of the lesions are removed (typical for products of alkylating agents) and site *b* is repaired at half the average rate. After repair, occupancy at site *b* is $p_{b/Pb} = (0.01)^{1/2} = 0.1$: a factor of 2 in repair rate leaves 10-fold more lesions than average at the slowly repaired site.

Preferential Repair of the Transcribed Strand. A DNA strand that is being actively transcribed is preferentially repaired by the *uvrABC* excision nuclease (chapter 121). The effect is to reduce mutations with the initiating lesion in the transcribed strand; for example, in the *E. coli lacI* gene mutagenized with UV, mutations were mostly at PyPy sequences (sites of photodimers) in the nontranscribed strand (133). In cells deficient in the factor that couples excision repair to transcription, mutations were mostly at PyPy sequences in the transcribed strand (133), in agreement with the observation that in the absence of the factor, RNA polymerase stalled at a lesion blocks nucleotide excision repair (161).

Mutation Frequency Decline The high UV-induced reversion rates for WP2(*trp)* and WU36(*tyr*) strains decrease by a factor of 10 in irradiated cells incubated under conditions such that protein synthesis does not occur (186). This effect, named mutation frequency decline (MFD) (44), occurs only for mutations in cells with nucleotide excision repair and (to date) only in tRNA genes (reviewed in references 162 and 189).

For certain C:G sites in tRNA genes at which UV-induced mutations showed MFD, those induced by ethyl methanesulfonate (EMS) did not show MFD; conversely, for a site at which EMS mutations showed MFD, UV mutations did not (17). Bockrath et al. (17) deduced that MFD involves selective nucleotide excision of initiating lesions from the transcribed strand: C-containing dimers at sites showing MFD of UV mutations, and O^6-ethylguanine at the site showing MFD of EMS-induced mutations. This conclusion was supported by the finding that an *mfd* mutant selected as excision competent but without MFD (187) lacks preferential excision from an actively transcribing strand (163) as discussed in the previous section. However, there is still a paradox (162, 189): although selective excision in a gene for a protein requires active transcription, MFD is observed when protein synthesis is blocked, presumably shutting down transcription of tRNA genes! A second complication is that UV-induced mutants showing MFD respond anomalously to photoreactivation (next section).

Mutagenesis Caused by Repair

For mutagenesis by physical or chemical agents, it is sometimes asssumed that the actual changes in DNA base sequence take place during semiconservative replication of the genome. There is, however, solid evidence that some of these changes occur during repair and before replication.

Mutagenesis by Nucleotide Excision Repair. Nucleotide excision repair is largely error free, since cells with such repair have both higher survival and lower mutation rates than excision-deficient cells given the same exposure to an agent making bulky DNA lesions. However, nucleotide excision repair can also cause changes in base sequence.

Photoreactivation (chapter 121) can be used as a tool to remove cyclobutane dimers in DNA in UV-irradiated *E. coli* at a known time. At short times after UV exposure, photoreactivation of excision-competent *E. coli* greatly reduces the number of mutants per survivor by removal of these dimers (thought to be the principal mutagenic photoproduct [79]) and then has a decreasing effect at longer times as shown schematically in Fig. 3; photoreactivation becomes ineffective when photoproduct excision is essentially over (as expected) and, in most cases, much before significant DNA synthesis (typically delayed as a result of UV exposure). For cells deficient in nucleotide excision repair, photoreactivation causes large decreases in mutants per survivor at long times after irradiation and loses effectiveness only after much of the DNA is replicated (Fig. 3). This pattern has been shown for forward mutations to streptomycin resistance (22, 131) and for reversion of mutations in the protein structural genes *trp* (132) and *tyr* (90) and is thought to be typical for most UV mutations. The simplest interpretation of these results is that base sequence changes occur during nucleotide excision, and no satisfactory alternative has been proposed. (UV-induced reversions that show MFD can be reversed by photoreactivation any time before DNA replication, even in excision-competent cells [90, 132].)

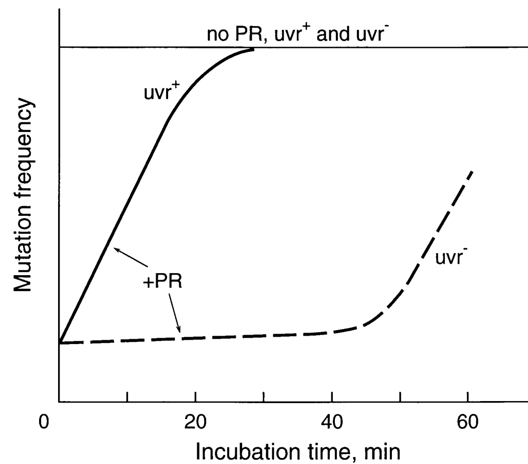

FIGURE 3 Effect on mutation frequency of photoreactivation (PR) after various incubation times following UV irradiation of *E. coli* uvr^{+} (excision-competent) and uvr (excision-defective) cells (schematic). See text for details.

Additional evidence for mutagenesis by excision repair obtained in studies of yeast cells is presented here for two reasons. It is of great importance that mutations can be fixed before replication of the genome, the process thought by many to actually change the DNA base sequence. Further, the mechanisms by which excision repair causes mutation are not established.

Diploid yeast cells in G_1 growth phase were irradiated with UV and allowed to divide once, and then recessive lethal mutations were determined by tetrad analysis; for cells without excision repair, the segregation pattern was that on the left in Fig. 4, whereas excision-competent cells showed the pattern on the right, implying that because of excision, a mutation exists in both strands of the DNA double helix before replication (82, 83, 89). In another experiment, *arg* or *lys* yeast cells were mutagenized and revertants were determined on plates either with minimal medium or with minimal medium supplemented with enough of the required amino acid to permit a few cell divisions. With UV as the mutagen, excision-competent cells showed comparable numbers of revertants per survivor on either kind of plate, so mutation was equally likely with little DNA replication or with several rounds; excision-defective cells showed significant reversion only with some DNA replication on supplemented plates (88). The conclusion is that changes in base sequence in yeast genes can occur before DNA replication as the result of nucleotide excision repair.

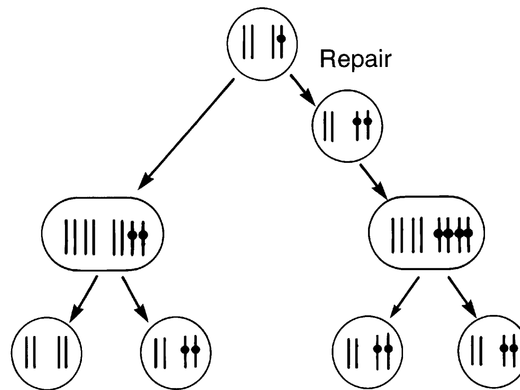

FIGURE 4 Effect of nucleotide excision repair on UV-induced mutagenesis in diploid yeast cells. Excision-proficient cells were irradiated and allowed to divide once; tetrad analysis of the daughter cells showed the genetic composition to be that on the right (mutation shown by a black dot), suggesting the pathway as shown (82). Excision-deficient cells gave daughter cells with the genetic composition on the left, showing that excision repair is required to form mutations in both strands of irradiated DNA before replication (83, 89).

A possible mechanism for mutagenesis by nucleotide excision repair is as follows. The *E. coli* UvrABC nuclease cuts out a single-stranded 12- to 14-mer oligonucleotide that includes the lesion (chapter 121); during replication to close the gap in the DNA, translesion synthesis over a second lesion in the template strand could cause a mutation. Naively, few pairs of lesions so near one another would be expected. However, closely spaced UV photoproducts, one in each strand, occur at a frequency about 1% that of all photoproducts (6, 95), in numbers that vary with the 1.2 to 1.4 power of the fluence (96), and are acted upon by the *uvr* nucleotide excision enzymes (177). Only a very small fraction of lesions cause mutations (see above), so observed mutation rates are not inconsistent with the abundance of closely spaced lesions. Pathways for repair of such closely spaced lesions have not been identified, but in addition to translesion synthesis, a reasonable possibility is the scheme for repair of psoralen cross-links between strands (chapter 121).

In excision-proficient cells, mutations could occur by excision repair, replication, or both. Note that repair of closely spaced lesions first excises selectively the lesion in the transcribed strand, and so will generate a mutation at the nearby lesion in the nontranscribed strand, in agreement with data on strand-selective repair.

Many more mutations, mostly C:G to T:A, were found after photoreactivation of UV-irradiated plasmids treated in vitro with a protein fraction (fraction II) from *E. coli uvr*$^+$ cells than with the fraction from *uvr* mutant cells; this mutagenesis did not depend on *umuC* or *recA* (30, 31). The relation of this process to in vivo nucleotide excision repair is as yet problematical.

Other Error-Prone Repair. Other kinds of repair also cause changes in base sequence. The clearest example is the repair of double-strand breaks in DNA. A number of experimental results (e.g., reference 33) show indiscriminate ligation of free DNA ends with each other, which can cause gross alterations in sequence such as deletions and inversions. Figure 5 illustrates a pathway for repair of double-strand breaks that is characterized by deletion of DNA between two direct sequence repeats, as well as one of the repeats; the mechanism is strongly supported by results of studies using *E. coli* and several other kinds of cells.

PATHWAYS FOR FORMING POINT MUTATIONS

Base Substitutions

The most common type of mutation is that in which one base pair has been replaced by another. Base substitutions are induced by mutagens mainly by translesion synthesis, with or without the assistance of SOS-associated proteins. The kind and site of base substitutions are governed by the lesion (Table 1). Tandem mutations such as CC to TT are induced primarily by mutagens that form lesions involving two adjacent bases on the same strand of the DNA double helix (76), such as UV [pyrimidine dimers or (6–4) products (136)] or *cis*-platinum(II) (cross-links between adjacent purines on the same strand [54]).

Some lesions do not provide the polymerase with any information concerning the original base and are called noninstructive, the prototype being the abasic site. There is evidence that the base just following the lesion influences the nucleotide inserted (92), suggesting that a base added while the lesion (X) is unstacked (N in Fig. 6b, lower diagram) moves opposite the lesion when the DNA changes to the upper configuration (dislocation mutagenesis). Some lesions have a low mutation rate (Table 1), suggesting they may usually be instructive. An interesting case is a common mutation induced by UV, C:G to T:A, by insertion of A opposite a C in the most frequent photoproduct, the cyclobutane pyrimidine dimer; C's in a cyclobutane ring can deaminate to form U's (62), so it is unclear if insertion of A is primarily a polymerase error or a correct pairing of an A with a U.

Most base substitutions change an amino acid in the gene product, so-called missense mutations. Some base substitutions do not change the amino acid and are silent: transitions in the third position of all codons (except TGG), and any base change in the third position of about half of all codons. A third type changes a triplet coding for an amino acid to one coding for a stop codon, a mutation suppressible by a tRNA suppressor (see above).

Frameshifts

Deletion or insertion of one or two base pairs, called a frameshift here, is the second most common type of point mutation. Most such frameshifts occur in monotonic runs of two or more of the same bases; frameshifts in runs of alternating bases, such as -GCGCGC-, are nearly always deletions or duplications of the repeating unit (-GC-). While a replication fork is blocked at a lesion, looping out of a base in a monotonic run could allow slippage of one strand with respect to the other (174), making a newly synthesized strand with one more or one less base (Fig. 6a). If translesion synthesis past the blocking lesion also introduces a base change, a frameshift with an accompanying base substitution, one of the more common complex mutations, is made. Unfortunately, data to support this suggested pathway for making a frameshift are still inconclusive, a generation after it was proposed.

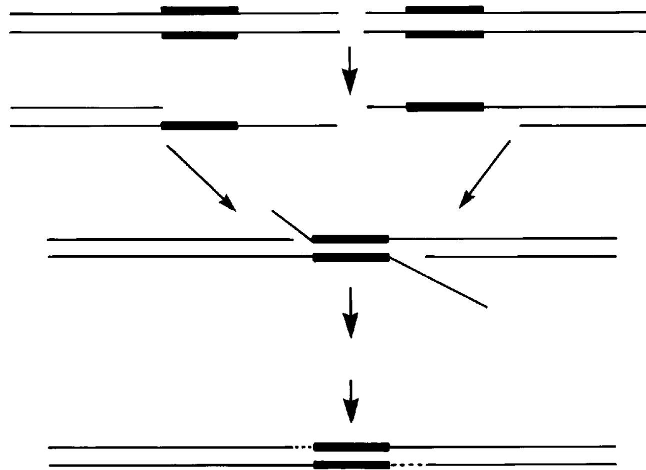

FIGURE 5 Repair of DNA double-strand breaks by single-strand annealing. Single-strand exonucleases digest strands (second line) to expose direct repeats (heavy lines), which anneal together (third line). After unpaired ends are removed, gaps are ligated (dotted lines) to complete the joining. There is strong evidence for this pathway in *E. coli* (32, 157), mammalian (146), nematode (139), and yeast (61) cells, as well as in frog oocytes (84).

There are several lines of evidence for a mechanism (Fig. 6b) making –1 frameshifts that could explain why mutagens damaging DNA produce more of these than +1 frameshifts. At a stalled replication fork, a base (N) is usually added fairly quickly opposite the lesion. Addition of another nucleotide to complete translesion synthesis is typically much slower, which gives an opportunity for the lesion to loop out (Fig. 6b); if the terminal base on the growing strand forms a Watson-Crick pair with the template base beyond the lesion, rapid extension is likely and the new strand is one base shorter.

Some compounds such as acridines induce in *E. coli* both –1 and +1 frameshifts in runs of identical bases, and essentially only frameshifts, by complexing with DNA and without necessarily causing damage (27, 171). It was originally suggested that acridines generate frameshifts by intercalating between stacked DNA bases (103). However, realistic molecular models for one-base deletions at 5′PyPu sequences induced by the acridine proflavine during in vitro replication by *E. coli* Pol I (Klenow fragment) suggest that these frameshifts arise from stabilization of a looped-out base sandwiched between two proflavines (16). While this model was not intended to account for other mutagenic processes, the findings do support the possibility that looped-out bases stabilized by acridines cause frameshifts in monotonic runs.

In coliphage T4, acridines induce frameshifts both in monotonic runs of bases (134) and at hot spots that are the sites of action of T4 topoisomerase II (145). This enzyme has similarities to mammalian topoisomerase II, and acridines may induce frameshifts in mammalian cells by the same pathway, but there is no evidence for frameshifts at the sites for *E. coli* gyrase (50), which is quite a different enzyme.

Complex Mutations

Two or more mutations in the same gene are observed fairly often, at a frequency far higher than could be expected from two or more independent events. Further, the individual mutations are not randomly distributed throughout the gene but located more closely together, suggesting that most are induced by a single initiating event. Mechanisms have been suggested above for two specific types of such mutants: adjacent base changes or tandem mutation (see the section on base substitutions), and a base change with an accompanying frameshift in a run of the same base (see the section on frameshifts). Excluding these, an additional 10 closely spaced (within a few base pairs) complex mutations in *E. coli* are in the Yale database

(77), of which two may arise from a process in which nearly inverted repeats become more exact inverted repeats (Fig. 7c; see the section below on strand switching and copy choice), a process suggested on the basis of similar mutations in phage T4 (40).

In addition, there are 70 other *E. coli* mutants in the Yale database with two mutations (nearly all base changes), and there are 3 with three base changes each, in various genes and induced by various agents. The separations range randomly from a few base pairs to a few hundred, with a mean of about 75. The mechanism is unknown.

GROSS DNA REARRANGEMENTS

Gross DNA rearrangements include large (hundreds of base pairs and more) deletions and insertions, inversions, translocations, and duplications. Among mutations induced by most mutagens, such rearrangements, deletions excepted, are found only at very low frequencies. Nevertheless, such rearrangements often involve a number of genes and can have a profound effect on the cell. They are covered in more detail in chapter 120. Transposition of mobile elements such as insertion sequence elements has been extensively studied (chapter 124). In one case, UV greatly increases excision of transposon Tn*10* (104).

Deletions

Much the most common large-scale alteration found among mutants in a particular gene is the deletion, ranging in size from 10 or so base pairs to hundreds of kilobases, a limitation set, presumably, by the requirement that the cell be viable.

Large deletions affecting two or more genes can be detected by genetic mapping and have been reported after treatment with ionizing radiation and with nitrous acid, at a frequency about 10% of that of mutations in a single gene: in the *his* operon in *S. typhimurium* (69), the *gal-chl* region of *S. typhimurium* (3), and the *E. coli lac* operon (158) (also in phage T4 treated with nitrous acid [179] and gamma rays [31a]). No such deletions were found among more than 100 sequenced mutations induced by gamma rays in the *c*I repressor gene in λ phage and prophage (180); possibly, deletions formed were not detected because they killed the phage. In an *E. coli* strain with over 100 kb of dispensable DNA surrounding a target gene, about 10% of gamma ray-induced mutations were deletions of 0.5 to more than 80 kb, centered around a ColE1 origin of DNA replication (141); the deletion frequency decreased by at least an order of magnitude when this origin was removed. Large deletions are apparently induced by agents that damage both DNA strands at closely apposed sites: ionizing radiation, which makes double-strand breaks and sites which are sensitive to *Aspergillus* S1 endonuclease (65), and nitrous acid, which forms interstrand cross-links (51). The frequency of large deletions in bacteria treated with most other agents is so small that it is unclear whether those observed are induced or of spontaneous origin.

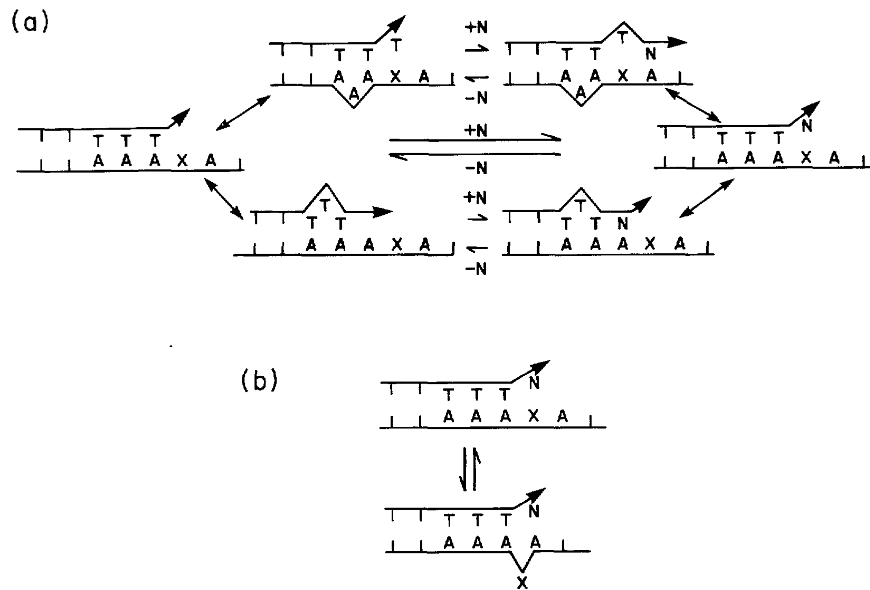

FIGURE 6 Possible pathways for frameshift mutagenesis at a replication complex blocked at a lesion (X). (a) Strand slippage (174). If a base on the strand being newly synthesized loops out, a +1 frameshift will occur; if the looped-out base is on the template, the result is a -1 frameshift. (b) Rearrangement of a newly added nucleotide (N) opposite a template lesion (92, 93, 97, 167, 183). Delay in adding another nucleotide can give time for the lesion (X) to loop out. If the terminal base (N) makes a Watson-Crick pair with the base beyond the lesion, the growing chain will be quickly extended.

Fragmentary information suggests two mechanisms for forming large deletions. At low doses, most deletions might be associated with specific DNA sequences (such as the ColE1 origin of replication; see above) and induced by a single lesion (75). For higher doses, and for genomic regions where very large deletions still give viable cells, most deletions probably are made by the classical two-hit mechanism: one end of a DNA double-strand break is misrepaired with an end from another break with loss of the intervening segment, a mechanism with strong support from data obtained from studies using *Neurospora crassa* (41).

Some papers report deletions ranging from tens to many hundreds of base pairs induced in genes on various plasmids in *E. coli* cells by agents such as UV (118, 155). Many such deletions eliminate both the DNA between two direct repeats 3 to 9 bp long, as well as one of the repeats (155), which suggests that the deletions arise from repair of double-strand breaks by single-strand annealing (Fig. 5). In the Yale mutational database (77) with more than 13,000 mutations in *E. coli,* all data sets with a significant fraction of these deletions are for genes on plasmids; none are for a gene on the chromosome. The implication is that plasmids may be more susceptible than chromosomes to formation of double-strand breaks and/or less likely to be repaired error free.

Template Switching or Copy Choice

Deletions, as well as other sequence changes, are sometimes interpreted in terms of a mechanism in which a replication complex is assumed to switch from one template to another or to another part of the same template, as diagramed in Fig. 7. In a formal sense, there is probably much truth in such strand switching/copy choice schemes. The mechanism implied by the term “strand switching/copy choice” and by the diagrams, however, has little experimental backing; two recent papers reporting data that supported copy choice mechanisms have been retracted (24, 56). There are, however, some in vitro data on the process shown in Fig. 7c (135). It seems likely that in most cases, results such as those diagramed in Fig. 7 are attained in quite different ways. The overall effect of postreplication repair (Fig. 1b) could

readily be described in terms of copy choice, although the actual mechanism is quite different. The process shown in Fig. 7a, deletion of one of two direct repeats and the intervening DNA, can occur by repair of a double-strand break (Fig. 5), for which there is a wealth of data.

MUTATIONAL SPECTRA

The number of different kinds of mutations and of the locations of these sequence changes is sometimes referred to as a mutational spectrum. Table 2 shows the spectrum of kinds of mutations induced by spontaneous processes in two *E. coli* genes. Mutational spectra induced by genotoxic agents differ enormously: methylating agents such as *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine induce nearly all G:C-to-A:T transitions in runs of two or more consecutive G's, whereas ionizing radiation induces nearly all kinds of mutations without any one dominant type. The observed spectrum depends on a complex array of factors: on the lesions in the DNA and the changes by repair and by decay of unstable species, on the mutagenic processes involved in replication and repair, and on the assay.

Lesions in the DNA

Lesions in the DNA depend not only on the mutagen but also on accessibility of the DNA (for chemicals) and on base sequence; for example, UV-induced dimers require adjacent pyrimidines in the same strand, and methylation by *N*-methyl-*N*-nitrosourea of G to make O^6-methylguanine is strongly dependent on flanking bases (123, 164). Some lesions are unstable and transform with time into other compounds (e.g., deamination of C in cyclobutane pyrimidine dimers) (62). Repair affects not only the total number of lesions but also the distribution in kind and location (see section above on repair and mutagenesis). The extent to which repair is expressed can depend on the treatment level, for example, as in the SOS response (chapter 89) or in the *ada* genes (chapter 121); specific repair pathways can be blocked by defects in repair genes such as *uvr* or *rec.*

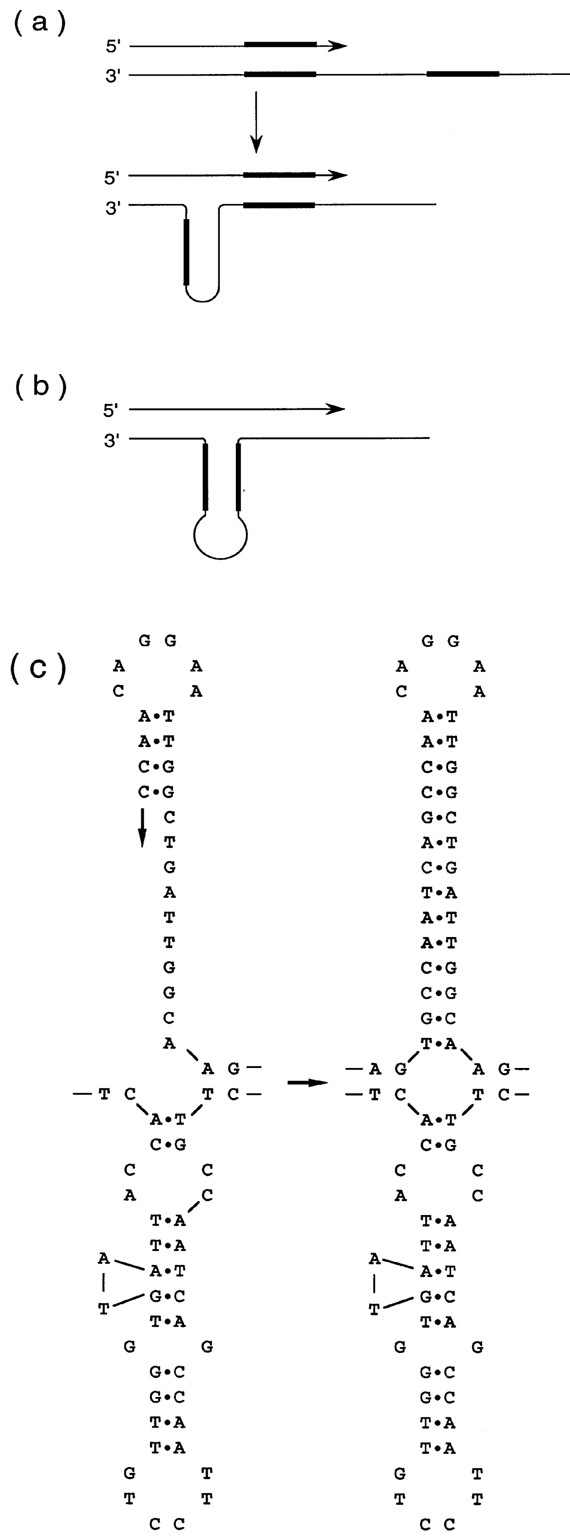

Figure 7 Strand switching or copy choice. See text for the reasons that the diagrams are thought to provide only formal representations and should not be taken as depicting actual mechanisms. (a) Deletion of DNA between two direct repeats (heavy lines), together with one of the repeats, by a growing polynucleotide chain mispairing with a downstream repeat as in Streisinger's slipped pairing (2). (b) Single-strand template DNA with two inverted repeats (heavy lines) forms a hairpin that allows

a replication complex to jump over to the other arm, deleting the hairpin (67). (c) A template with inverted pseudorepeats goes into a hairpin and allows the replicating strand to fold back and copy itself, forming exact inverted repeats (40).

TABLE 2 Spontaneous mutation spectra in genes in *E. coli*[a]

Reference for data set	Gene	No. of:												
		Mutations	Transitions		Transversions				Frameshifts		bp 620 ±TGGC hot spot	Deletions	Duplications	Insertion sequences
			C→T	T→C	C→A	C→G	T→A	T→G	–N	+N				
154	*lacI*[b]	414	137	38	23	12	35	48	18	ND		71	31	1
153	*lacI*	172	6	3	3	0	3	5	4	1	117	22	3	7
68	*lacI*	729	45	5	13	3	7	9	28	7	525	72	7	8
59	*lacI*	140	←			(24 point mutations)				→	94	19	1	2
170	λ cI	52	14	5	5	1	7	2	5	3		1	0	9

[a]C → T indicates a C:G-to-T:A base change, etc.
[b]Only *lacI* mutations in bp 30 to 242 were detected by the assay used, which excluded the ±TGGC hot spot at about bp 620. In this assay, +1 frameshifts are not detected (ND) (154).

Mutagenic Processes

The probability that a lesion at a replication fork induces a mutation, and the kind of mutation, depends on competition between mutagenic translesion synthesis and nonmutagenic postreplication repair. An additional contribution comes from repair processes such as nucleotide excision that change the base sequence in the process of eliminating the lesion.

Assay

Mutation assays usually depend on alterations in gene product activity and detect with high probability those mutations, such as frameshifts, deletions, or stop codons, that make large changes. However, the most common mutation is a base substitution that changes an amino acid, which can affect activity by a little or a lot, and may or may not be detected in a given assay, particularly in a gene with a strong promoter (101).

Valuable information has been obtained from mutational spectra about mechanisms of mutagenesis and identification of mutagenic agents. Several statistical methods for analysis are available (1, 13, 138). Any such analysis, in addition to purely statistical considerations, must take into account the many and sometimes subtle biological effects that influence these spectra.

SPONTANEOUS MUTAGENESIS

Frequency and Spectrum of Spontaneous Mutations

The mutation frequencies and spectra for untreated cells that are given in most papers are most accurately thought of as background: useful, certainly, for comparison with the same parameters after mutagenic treatment, but dependent on the history of the cultures and frequently differing greatly from one experiment to another. Therefore, care must be taken in drawing conclusions regarding spontaneous mutagenesis from such published data.

A meaningful spontaneous mutation frequency and spectrum can be determined by starting with several cultures so small that there is little chance of a preexisting mutant and allowing each to grow to a known size N under defined conditions that do not discriminate between mutated and unmutated cells. The mutation rate per replication ϕ is given by $\phi = 0.4343\, f/\log(N\phi)$, where f is the median of the measured mutation frequencies (46), assuming that phenotypic lag is small. (Note that the equation must be solved by iteration.) A mutation that occurs early in growth (or is in a culture initially) gives rise to a

"jackpot" with a high observed mutation rate, so a grossly outlying value should be excluded. In determining the spectrum of spontaneous mutations, allowance must be made for a mutation that occurs late enough that the overall mutation frequency is not substantially affected but early enough that the mutated cell divides a number of times, producing an apparent mutational hot spot. The usual procedure is to keep only one of two or more identical mutations from a particular culture, unless the same hot spot appears in several independent cultures.

A plausible estimate for the spontaneous mutation rate in growing *E. coli* cells is about 0.003 mutation per genome per DNA replication, based on data for 12 DNA-based microbial genes (including three *E. coli* and four coliphage genes) (46); the figure is the total estimated mutation rate and includes corrections for undetected base changes (base substitutions that do not change the amino acid and missense mutations that do not sufficiently change gene product activity). This is about 6×10^{-10} mutations per base pair, or about 6×10^{-7} mutations in a typical 1,000-bp gene, per DNA replication.

The available data on the spectrum of spontaneous mutations in *E. coli* are summarized in Table 2. In the *lacI* gene, about two-thirds of the mutations are loss or gain of TGGC, an instability presumably linked to the rather unusual multiple repeats of that tetranucleotide in the gene sequence. On the basis of the data for the two genes listed, it appears that for base changes, C:G-to-T:A transitions are the most frequent and C:G-to-G:C transversions are about 10-fold less common; the other four base changes are roughly one-third to one-fourth that for C:G to T:A. Single-base frameshifts occur about one-third to one-fourth as frequently as the sum of all base changes, with deletions more common than additions. There are great differences from one data set to another in occurrence of deletions and duplications (excluding the *lacI* hot spot); possibly the location of the *lacI* gene on an F′ episome in these experiments contributes to this variability. The greatly disparate rates of IS insertion sequences arise presumably from the number and location of such elements in the genomes of particular strains used in the various experiments (see chapter 124).

Mutagenesis from DNA Lesions in Untreated Cells

DNA in cells not treated with exogenous agents is damaged in a number of ways (105), so some spontaneous mutations are formed by pathways similar to those induced by mutagens.

DNA Hydrolysis. Sites of 5-methylcytosines are hot spots for spontaneous mutagenesis in *E. coli* (36), because loss of the amino group by hydrolysis forms T, and the resulting G:T pairs are apparently less efficiently repaired than most mismatches. Cytosine also loses its amino group by hydrolysis, three- to fourfold more slowly than 5-methylcytosine, but the resulting uracil is removed almost completely by uracil-DNA glycosylase, so this process is significantly mutagenic only in *E. coli ung* cells deficient in this enzyme (53). Levels of deamination of adenine to hypoxanthine (a product excised by 3-methyladenosine-DNA glycosylase [151]) and of guanine to xanthine are small compared with that of cytosine and seem to be unimportant.

The *N*-glycosyl bond between the deoxyribose sugar and a base is subject to acid hydrolysis at a rate the order of a few purines per *E. coli* genome per day at 37°C, G being released at 1.5-fold higher rate than A and the pyrimidines being released at a rate only 5% of that of the purines (105). The resulting abasic sites are quickly cleaved to single-strand breaks, and since these are added to the much larger number of such breaks formed by a variety of processes which are efficiently repaired, base hydrolysis is not normally a significant source of mutation.

Attack on DNA by Oxidizing Species.

Normal metabolic processes in *E. coli* in the presence of oxygen lead to the production of highly reactive oxidizing species: hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), and hydroxyl radical ($OH^\bullet$)(see chapter 95). These in turn can react with DNA to oxidize bases and make strand breaks.

The product that has received the most attention is 8-oxo-7,8-dihydroguanine (8-oxoG) (Fig. 2), which pairs preferentially with A to make a transversion (168). The most reliable figure now available for the number of 8-oxoG molecules in DNA is $2.8/10^6$ guanines, in *E. coli* (M. Sekiguchi, personal communication); much higher values reported by others probably reflect oxidation occurring during DNA isolation (105). The mutagenic potential of 8-oxoG is such that three mutator (*mut*) genes in *E. coli* have been identified as coding for enzymes that act to reduce the level of this product in DNA (178): *mutM* is the *fpg* gene for the glycosylase that removes 8-oxoG, as well as the ring-open forms of oxidized purines (formamidopyrimidines), from DNA; *mutT* is the gene for an enzyme that degrades 8-oxodGTP and thus prevents its incorporation into DNA; *mutY* is the gene for a specific glycosylase that excises A paired with G or 8-oxoG in DNA.

The most frequent oxidized product of A is 8-oxoA, but this product largely pairs with T and thus is not mutagenic (166). For T, the most common product is thymine glycol, which is a block to some polymerases and thus can be toxic but is relatively nonmutagenic because the base usually inserted opposite is A (11, 70). Attack of oxidizing species on C in DNA gives comparable quantities of 5-OH-C, 5-OH-U, and uracil glycol (181); the 5-OH-pyrimidines mispair in vitro, leading to C:G-to-T:A transitions and C:G-to-G:C transversions (140). Data have been presented for a relatively large number of CC-to-TT mutations induced by reactive oxygen species in single-stranded DNA assayed in *E. coli* (143), but there is no evidence for such tandem base changes in cells with double-stranded DNA mutagenized by a variety of oxidizing agents (76).

Other Agents Damaging Intracellular DNA. The widely occurring metabolic intermediate S-adenosylmethionine can transfer a methyl group to purines (149), but the products, 3-methyl-adenosine and 7-methylguanine, are relatively nonmutagenic; the very mutagenic O6-methylguanine and O4-methylthymine do not seem to be made by this pathway (105). All living cells are exposed to ionizing radiation from naturally occurring radioisotopes and cosmic rays, with an intensity the order of 0.1 rad/year. However, E. coli cells are poorly mutable by ionizing radiation: a single dose of 10,000 rads induces about 10–5 mutations in a typical gene, so mutagenesis from background radiation is small compared with that from the endogenous oxidizing species discussed above.

Enzymatic Attack on Intracellular DNA. DNA(cytosine-5)-methyl- transferases, which normally transfer a methyl group from S-adenosylmethionine to cytosine, can catalyze deamination of C to U at low concentrations of *S*-adenosylmethionine (165), as might happen in starving cells.

Mutagenesis from DNA Replication Errors

Polymerases make errors in replicating the genome (chapter 50); a number of mutator genes identified phenotypically in *E. coli* turn out to be genes for products that govern the faithfulness of DNA replication. There are three major components determining fidelity in DNA replication.

Base Selectivity of the Polymerase. The polymerase in the Pol III holoenzyme that replicates the *E. coli* genome is the product of the *dnaE* gene. Various factors govern the selectivity with which the polymerase chooses a base to pair with the complementary base in the template (55), and mutations in the polymerase gene make enzymes with both higher and lower error rates (48, 60). In wild-type *E. coli,* errors in base selection made by the polymerase in the Pol III holoenzyme occur about once in 500,000 bases (152).

Proofreading. In the Pol III holoenzyme, an editing 3′→5′ exonuclease, product of the *dnaQ* gene, removes bases from the growing strand that do not pair with the base in the template (chapter 50). The powerful *mutD* mutator has been identified with defects in the *dnaQ* gene, and such strains can have mutation frequencies up to 10,000-fold more than wild-type frequencies. Of this value, only a factor of 100 comes from loss of editing; this leads to so many mismatched bases that the *mutHLS* mismatch repair (see below) is saturated (125), accounting for the high mutator activity in the *mutD5* phenotype.

Mismatch Repair. The mutator genes *mutH, mutL,* and *mutS* map in genes for three mismatch repair proteins, and *mutU* maps in the gene (*uvrD*) for helicase II; these proteins correct mismatched bases and frameshifts of one to three base pairs in the DNA just behind the replication fork (125) (chapter 121). Mutations in the *dam* gene for the enzyme methylating adenine cause loss of the methylation signal that guides mismatch repair (chapters 53 and 121) and also has mutator properties. Overall, mismatch repair reduces errors by a factor of 200 or so (152).

Polymerase selectivity, proofreading, and mismatch repair account for an overall replication error rate the order of 10^{-10} per base (152), which can be compared with the estimated total mutation rate for *E. coli* of 6×10^{-10} per base pair (46) (see above). The uncertainties in these numbers are so large that no realistic estimate can be made of the fraction of spontaneous mutations which arise from mistakes in replication and the fraction from damage in the DNA, but it seems likely that both sources of error contribute substantially.

Spontaneous Gross DNA Rearrangements

Such mutations as deletions, mobile DNA elements, duplications, inversions, and transpositions are found more commonly in mutants of spontaneous origin than among those induced by exogenous agents. Mobile DNA elements are discussed elsewhere (chapter 124) and other gene rearrangements are also treated in more detail elsewhere (chapter 120). Many occur between direct or inverted repeats of a few or a few hundred bases: deletions involving homologous recombination between long (8) and short (185) direct repeats; inversions between short inverted repeats (156); and tandem duplications between regions of homology, with a frequency of 10^{-5} to 10^{-3} per cell in the *E. coli* chromosome (5). The cited papers give results suggesting that recombinational mechanisms are involved (but see reference 113).

Mutator Genes

A number of genes in *E. coli* that are important in mutagenesis were first identified because their mutator phenotype gave substantially increased levels of spontaneous mutagenesis. Since the functions of products of theses genes are now more or less understood, they are best discussed during the systematic description of mutagenic mechanisms, as described above; for the convenience of the reader, the more widely studied *mut* genes are listed in Table 3, together with brief notations about function.

TABLE 3 Common mutator genes in *E. coli*

Mutator gene	Specificity	Effect
mutD	Base changes and frameshifts	Altered proofreading of Pol III holoenzyme
mutH *mutL* *mutS* *dam* *uvrD*	Base changes (mainly transitions) and frameshifts	Defective mismatch repair
mutM	G:C to T:A	Defective formamidopyrimidine (8-oxoG) glycosylase
mutY	G:C to T:A	Defective glycosylase excising A paired with G or 8-oxoG in DNA
mutT	T:A to G:C	Defective enzyme degrading 8-oxodGTP

SPONTANEOUS MUTAGENESIS IN SOS-INDUCED CELLS

Induction of the SOS response in *E. coli* cells increases the level of spontaneous mutagenesis. If untreated λ phage are assayed in host cells that have been irradiated with UV, the number of mutant plaques can increase by 100-fold (42, 81). Increases in spontaneous mutation are found in cells in which the SOS response has been induced genetically by incorporation, for example, of the *recA441*(*tif)* gene (121, 190), or when an F′ episome is transferred by conjugation from an unirradiated to an irradiated cell (29, 94).

One mechanism for this increase in mutagenesis with induction of the SOS response is, with reasonable certainty, SOS-aided translesion synthesis, with the aid of the UmuC-UmuD′-RecA complex, of damage sites of spontaneous origin (such as abasic sites), producing mainly base changes (121).

A second mutagenic mechanism dependent on SOS induction is shown by untreated λ phage assayed in irradiated host cells. There is a great increase in the number of bursts with mutant phage, and nearly all the bursts have only one or two mutant phage among a normal number of wild-type phage. The numbers of mutants per burst are consistent with mutagenesis arising from a modest loss of fidelity in replication, which is amplified by the many rounds of phage DNA synthesis (80). The mutations induced are largely frameshifts in runs of the same base (193). The process does not occur in *uvr* mutant cells (42, 110) or in cells with an insert in the *dinB* gene (23), a gene of unknown function that is part of the SOS regulon (chapter 89); even in cells in which the SOS genes (including *dinB*) are constitutively turned on, UV irradiation of the cell is still required (23). Finally, this second mechanism functions in *umuC* (109, 193) and *recA* (23) cells in the same way as in wild-type cells. One speculation is that nucleotide excision repair of the host cell DNA sequesters some protein(s), which in turn causes a loss of fidelity of replication of the phage DNA.

This second mechanism may be a true example of "untargeted mutagenesis" (190). The first mechanism discussed above, of SOS-dependent spontaneous mutagenesis by translesion synthesis past lesions of endogenous origin, is targeted.

Inducing the SOS response by introduction of the *recA730* or *recA441* gene increases the number of large (hundreds or more base pairs) duplications by an order of magnitude, in a process that does not depend on *umuC* (43). The increase in such duplications by UV irradiation is about the same (73), suggesting that the principal effect of UV is SOS induction. Also, Tn*5* transposition is reportedly enhanced in constitutively SOS-induced *recA441* cells (91).

STATIONARY-STATE MUTAGENESIS

A paper published in 1988 (26) described experiments in which *E. coli* cells with a mutation in the pathway for lactose metabolism were spread on agar with lactose as the sole carbon source. Over a period of a number of days, there was a steady increase in colonies of revertants able to utilize lactose; the significant feature is that mutations not selected for, such as valine resistance, did not increase in the lawn cells. The basic observation has been confirmed a number of times in *E. coli* (63): in cells incubated for periods of up to 20 days in stationary phase, mutations that allow cells to resume growth are recovered at a very much higher rate than are mutations not being selected for. There is evidence for a similar result in yeast cells (63).

The phenomenon is important to evolution and the ability of cells to accommodate to adverse conditions, which has led some to name it "adaptive mutagenesis" (63). The top priority now is for insight into the mechanism, which should determine its scope (could it help nondividing mammalian cells become rapidly dividing cancer cells?) and ascertain if new mutagenic processes are involved; a number of the latter have been proposed, but so far all have been refuted (63). A significant difficulty is that the phenomenon is found only in cells well into stationary phase, which has received much less attention than rapidly growing cells (but see chapters 93 and 106 in this volume).

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