Mutagenesis

Secondary article

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Mutations have a potential for either positive or negative effects on an organism. To combat the deleterious effects of excessive mutagenesis, elaborate systems of enzymes have evolved to correct errors that arise spontaneously in cells or induced following DNA damage. However, DNA must necessarily change as organisms evolve, thus, some low level of mutagenesis is tolerated perhaps even promoted to assure normal and healthy levels of genetic variation of populations.

Article Contents

Introduction

Fundamental questions concerning the nature of mutations, mechanisms for mutagenesis and the factors that control rates of mutagenesis have been at the centre of intense study over the past several decades. Researchers in mutagenesis have made great strides in understanding the origins of mutations and their effects on broad biological processes such as carcinogenesis and evolution. In this article a number of methods and strategies that allow the detection of rare mutagenic events in prokaryotic and eukaryotic systems are surveyed. Molecular mechanisms for mutagenesis are described, with special emphasis on the mechanism for enhanced mutation rates associated with SOS induction in bacterial systems.

Mutations and Mutant Definitions

Researchers from a number of disciplines (e.g. genetics, physics, biochemistry, molecular biology and radiation biology) have made valuable contributions to our current understandings of mutagenesis, at the same time developing an extensive and 'rich' vocabulary to describe repair and mutagenesis. Because these terms may be unfamiliar or even confusing, an attempt will be made to keep them at a minimum and offer definitions as they are encountered.

The term mutation refers to any change in the genetic material (DNA) that is heritable. The normal sequence prior to the introduction of a mutation is referred to as wild-type, the form found in nature or in the 'wild'. However, 'wild-type' is not easily defined, since in nature there are many individuals within populations of the same species with significant genetic variation. Thus, the designation of wild-type is usually an arbitrarily chosen lineage that can be used as a reference.

Mutations can be grouped by a number of criteria that range from descriptions of the physiological consequences of a mutation (i.e. phenotype) to systematic molecular notations. Although descriptions of the phenotypes (e.g. dominant, albino, temperature-sensitive, etc.) can be informative, they can also become cumbersome. Extensive lists of nucleotide sequence changes (e.g. C102 to T transition in gene '*X*') are precise designations but also can become tedious and meaningless unless described in the appropriate molecular genetic context. For the purpose of this article, it is most useful to group mutations on the basis of DNA sequence alterations, but to limit this discussion to general concepts and classes of mutations. This article will also compare the prominent mutant classes with regard to sequence changes, mutational mechanisms and will relate the evolutionary significance of each class.

Mutations can be conveniently classified into two broad groups: (1) point mutations, which are often relatively subtle alterations involving one or few nucleotide changes and (2) rearrangements, which are more extensive or 'severe' chromosomal alterations involving segments of hundreds or even millions of nucleotides.

Point Mutations

The simplest point mutations are single-base substitutions, which arise when the wrong base has been inserted in the place of the correct one; they are subcategorized as transitions and transversions. Transitions are the simplest form of mutation, involving substitution of one pyrimidine for another or one purine for another purine. Transversions are also relatively uncomplicated, but involve switches of one base type for another type (e.g. a pyrimidine to a purine) **Table 1**).

The effect of a given substitution depends upon the informational context of that mutation. For example, a base substitution 'outside' of a gene may not produce a mutant phenotype and can be referred to as a silent mutation. Silent mutations can also be observed within genes. Some nucleotide substitutions within a three-base codon do not change its coding. For example, a substitution in an AGA codon to AGG would be a silent mutation since both of these nucleotide triplets code for the same

Table 1 Point mutations

Single base substitution								
Transitions	Pyrimidine → Pyrimidine (T→C or C→T)							
	Purine → Purine (A→G or G→A)							
Transversions	Pyrimidine → Pyrimidine (e.g. T→A)							
	Purine → Pyrimidine (e.g. G→T)							
Frameshift mutations: ± (3*n* ± 1)								
Example: –1 frameshift[a]	ACA	AGT	CGT	CCA →	ACA	GTC	GTA	CA
	Thr	Ser	Arg	Pro...	Thr	Val	Val...	
		(wild-type)				(mutant)		

[a]Four wild-type codons of a gene are shown and the amino acid coded by each is indicated below. The removal of a base (underlined) results in a change in the coding of adjacent sequences, producing a highly altered protein.

amino acid, arginine. Although silent mutations can occur both inside and outside of genes, the probability of a nucleotide substitution producing a mutation with phenotypic consequences greatly increases within genes.

A base substitution within the protein-coding region of a gene will often result in miscoding of an amino acid residue thereby producing a mutant protein. Some substitutions will produce totally defective mutant proteins and are called null mutations. However, the effect of an amino acid substitution may be more subtle, producing a mutant protein with a partial loss of activity, or occasionally an increase in activity. for example, single-base mutations are thought to be essential in the development of new species; mutant changes can be subtle enough to fine-tune a process, possibly making it more efficient in a given situation (Friedberg *et al*., 1995; Snyder and Champness, 1997).

Frameshift mutations occur within a protein coding region of a gene; these mutations arise from additions or deletions of one or few bases that are not multiples of three (most commonly ± 1, ± 2, ± 4, or ± 5 bases). Frameshift mutations are characteristically the most severe form of point mutation because they almost always produce null mutations. All of the codons that are downstream (i.e. distal) of the frameshift mutation will be out of register and thus miscoded **Table 1**). The miscoded region is typically so different from the normal gene sequence that frameshift mutations usually render mutant proteins completely nonfunctional.

Mutations that inactivate genes can also be changed back (or reverted) to restore the full function. The probability of a reversion mutation is usually related to the complexity of a mutation. For example, the simplest classes of sequence changes such as transition mutations are more likely to be reverted than other more extensive chromosomal alterations such as deletions (see below).

Sequence Rearrangements

Rearrangements are more drastic sequence alterations than point mutations. Rearrangements can involve only a few bases (∼10) or large segments of chromosomes involving millions of base pairs. These dramatic rearrangements are categorized into four general subclasses: deletions, inversions, translocations and duplications (**Table 2**). Many of these rearrangements are thought to be produced by the aberrant operation of the recombination, repair or replication enzymes of the cell and can also be produced in response to DNA damage.

Deletions remove segments of DNA. These can result in the loss of substantial segments of chromosomes including one or many genes. A deleted gene would not only be a null mutation, but since this DNA cannot be regained by a subsequent mutational event, it cannot be reverted. Deletions occur at surprisingly high frequencies in a number of organisms. Because of this relative abundance, it has been suggested that deletions may play a significant role in creating genetic diversity (Mahan and Roth, 1991; Miller, 1992; Snyder and Champness, 1997). Inversions

Table 2 Rearrangements

Rearrangement classes	Change[a]
Deletion	abcdefg→ab-fg
Inversion	abcdefg→ab-edc-fg
Translocation	abcdefg→ab-WXYZ[b]-cdefg
Duplication	abcdefg→abcdefg-abcdefg

[a] Letters indicate intervals of genes on double-stranded DNA.
[b] Uppercase letters represent a DNA segment that has been inserted into a new location. Each rearrangement produces a 'novel junction' (-) which connects two segments of DNA that are not ordinarily attached. The placement of a segment next to a different segment may have phenotypic consequences, such as altering gene expression.

and translocations are two additional classes of chromosomal rearrangements, but they do not necessarily yield a net loss or gain of DNA. Inversions result from 'flipping' the order of a chromosomal segment and, as a result, all genes in that segment are placed in the opposite orientation with respect to the rest of the chromosome. Translocations are mutations in which a fragment of DNA has moved from one chromosomal location to another. Mutagenic events that produce either inversions or translocations seem to be relatively rare compared to deletions (Mahan and Roth, 1991). Duplications are rearrangements that produce a net gain of DNA. They are defined as the formation of additional copies of chromosomal segments. Duplications occur at a moderately high rate and are thought to play an especially important role in evolution (Mahan and Roth, 1991; Miller, 1992). The presence of multiple copies of a gene through duplication allows the occurrence of mutations in the 'extra' copy without deleterious consequences because the 'primary' copy can remain unchanged and thus continue to code for a fully functional gene product (Snyder and Champness, 1997). The accumulation of multiple point mutations in duplicated copies could eventually produce a divergent gene product(s), allowing organisms to generate new diverse functions. The process of gene duplication and then divergence is widely believed to be essential in the evolution of species with greater complexity.

A special class of rearrangements are caused by transposable genetic elements, or transposons. These 'jumping genes' are segments of DNA that usually code for enzymes that promote their own movement. Transposable elements have been identified in numerous organisms, including bacteria, lower eukaryotes, insects and many mammals. Although usually rare, the movement of transposable elements can promote a variety of chromosomal rearrangements and are considered to be a driving force for evolutionary change.

Spontaneous Mutagenesis

Naturally occurring or spontaneous mutagenesis refers to genetic alterations that occur without apparent exposure to agents that damage DNA. Most spontaneous mutations are thought to be produced from a combination of replication errors and spontaneous damage to DNA. Nucleotides are occasionally misincorporated by a DNA polymerase (DNA pol) producing mismatches in the newly synthesized DNA strand (i.e. nascent strand). A number of cellular DNA polymerases, such as the major replicative polymerase of *Escherichia coli* (DNA pol III) have the capacity to stop DNA synthesis following a wrong base insertion and then remove that mismatch (**Figure 1**). The removal of mismatches is called proofreading and is achieved by a 3′→5′ exonuclease activity of DNA pol III, excising a portion of the nascent strand including the mismatch. The DNA pol then resumes synthesis of the nascent strand. The accuracy afforded to DNA pol III through base selection and proofreading is impressive, resulting in only one error per 10^5 to 10^6 bases replicated. However, even this high standard of accuracy is insufficient and could not be tolerated by an organism. Thus, there are a number of additional cellular surveillance systems that identify and remove mismatches. Due to the collective effects of the different editing/surveillance mechanisms, organisms like *E. coli* can maintain a genetic stability in which mistakes occur about once in 10^{10} replicated bases (Friedberg *et al*., 1995).

DNA present in cells has a finite chemical stability that is largely a reflection of the spontaneous chemical and physical damage that it suffers. To keep spontaneous mutagenesis to a minimum, there are a number of repair systems scanning chromosomes for the more common forms of damaged bases. If a damaged base is not removed before DNA pol encounters that site on the template, mutations can arise. Deamination of bases is relatively frequent and is potentially one of the most mutagenic forms of spontaneous damage. For example, deamination of cytosine will produce uracil, which during DNA

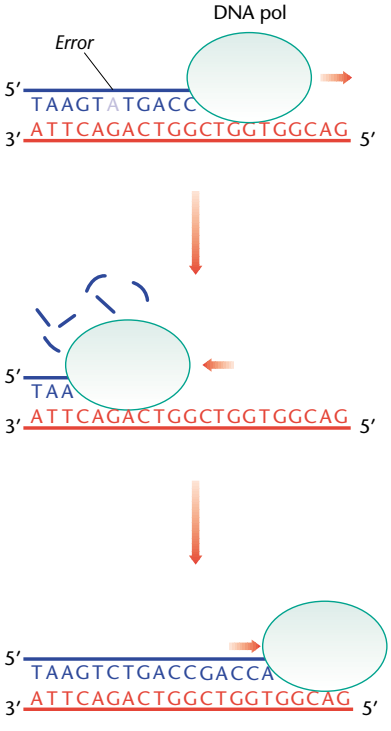

Figure 1 Proofreading activity of DNA polymerase. Following an incorrect insertion of a nucleotide ('Error'), DNA pol stops, reverses direction (3′→5′) and removes a portion of the nascent strand (top blue strand) that includes the error as well as some of the surrounding nucleotides. Following removal of sequences, synthesis of the nascent strand resumes.

replication will code for the addition of adenine (A) in the nascent strand. Such events would result in C to T transition mutations (or G to A in the complementary strand). In this example, the polymerase does not make a mistake; instead the change from cytosine to uracil presents misinformation to the DNA pol (Friedberg *et al*., 1995). Oxidative damage to DNA is another major cause of spontaneous mutagenesis. Reactive oxygen species such as hydrogen peroxide and superoxide are continually being produced as byproducts of normal cellular respiration. A failure to repair oxidative damage also contributes to spontaneous mutagenesis.

Many spontaneous mutations also result from insertions by transposons. When a transposon hops into a gene, the insertion of this large DNA segment will often disrupt the linear continuity of the gene, yielding a null mutation. The level of spontaneous mutagenesis due to transposition is highly variable and usually reflects the type of transposon(s) present in that organism.

Induced Mutagenesis

Mutation rates can be increased by conditions that damage DNA. Any chemical or physical agent that increases mutagenesis is referred to as a mutagen. Mutagenesis induced by exposure to damage is defined as induced mutagenesis. Mutagens introduce some chemical change to DNA such as altering bases or perhaps breaking the sugar–phosphate backbone. A damaged base or segment of DNA is not actually a mutation; it is instead referred to as a premutational lesion. The formation of a mutation usually depends upon the aberrant operation of some cellular process (e. g. DNA replication or recombination) after encountering a premutational lesion (Friedberg *et al*., 1995: Snyder and Champness, 1997).

Various types of radiation are mutagenic. X-rays produce breaks in the DNA and attempts to repair these breaks by recombination can lead to dramatic genetic changes such as deletions, translocations and other chromosomal rearrangements. Ultraviolet (UV) radiation catalyses the joining of adjacent pyrimidine bases, and these joined bases, or dimers, usually result in point mutations. Many of the early systematic molecular analyses of DNA damage and mutagenesis used UV, and our understanding of its effects is probably the most extensive. The most abundant UV photoproducts are pyrimidine cyclobutane dimers. All four possible dimer combinations are produced (T-T, T-C, C-T and C-C) but T-T dimers are the most common. Each cyclobutane dimer acts as an obstacle causing a DNA polymerase to stall, although occasionally the DNA pol continues synthesis past the dimer and will insert the wrong base (see below). The most prominent class of UV-induced mutations are transitions, followed by other types of point mutations. Stalling by DNA pol at UV photoproducts (as well as other related lesions) will induce a cellular DNA-repair process. In bacteria it is called the SOS response involving the increased expression of a number of genes whose products not only assist the cell to survive DNA damage but also increase mutation rates (Friedberg *et al*., 1995; Frank *et al*., 1996, and see below).

Chemical mutagens are categorized into four general groups, based on the mechanism by which they interact with DNA (Snyder and Champness, 1997). (1) Base analogues are structurally similar to bases; they have their mutagenic effect by being incorporated into DNA and causing mispairing during replication. (2) Intercalating agents are generally flat molecules that can fit between bases, producing helix distortions that can lead to replication errors. (3) DNA-reacting chemicals, such as reactive oxygen, can directly modify bases, changing coding groups and thereby allowing base pairing with the wrong base. (4) Alkylating agents bond covalently to DNA and result in the addition of some organic group to the bases or possibly to the sugar–phosphate backbone. The alkylating groups range widely in size and produce mutations by various mechanisms. The addition of small alkyl groups may modify the coding of a base and thus present misinformation during DNA replication. Bases altered by large bulky groups usually do not exhibit their mutagenic potential by misinformational replication; instead, DNA polymerase often stalls at these modified bases. These bulky adducts act as potent inducers of the SOS response (Friedberg *et al*., 1995; Frank *et al*., 1996). The induction of SOS can lead to the error-prone replication past a range of premutational lesions (see below).

Systems to Detect and Analyse Mutations

Typically, mutations are relatively rare, and it would be necessary to search through thousands, or even millions of individuals to detect a single mutation. This approach is sometimes referred to as a brute-force screen and is generally considered to be impractical. Because of this a number of more practical approaches have been devised to detect rare genetic changes. The most powerful approach depends upon the direct selection of mutants, taking advantage of lethal substances (i.e. toxins, antibiotics, viruses, etc.) that can kill an entire population except for those rare mutants resistant to a given substance (Miller, 1992; Friedberg *et al*., 1995; Snyder and Champness, 1997). For example, early mutagenesis studies used potent viruses that rapidly killed the bacterial host, allowing researchers to select rare mutants resistant to a particular virus (10^{-6} to 10^{-8}).

Most assays are classified as either forward mutagenesis or reversion mutagenesis assays. Forward mutagenesis refers to mutations that inactivate a functional gene; reversion mutagenesis, is essentially the reciprocal event, in which a mutation restores the normal function.

Forward assays detect a diverse spectra of changes since mutations that disrupt a gene are in every class (e.g. transitions, frameshifts, deletions, etc.) and occur at numerous locations within the gene. Early in the study of bacterial genetics, mutagenesis assays were developed that take advantage of both selection and forward mutagenesis of the *lacI* gene. The *lacI* gene codes for the repressor of the lactose utilization operon (*lacZYA*) of *E. coli*, and null mutations lead to the continual (i.e. constitutive) expression of that operon. A selection was based on the property that *lacI*$^-$ mutants could utilize a synthetic sugar (phenyl-β-D-galactoside) and grow because the *lac* operon was expressed constitutively (Miller, 1992; Snyder and Champness, 1997).

To clarify what types of mutations occur under different conditions, researchers have isolated a large number of mutants in well-characterized genes (called mutagenic targets) and have determined the DNA sequence changes for each mutant. Collectively, nucleotide sequence studies have revealed that all classes of point mutations are well represented, transitions usually being the most abundant, followed by transversions (Miller, 1992; Friedberg *et al*., 1995). One explanation for the relative abundance of these single-nucleotide substitutions is that the most subtle replication errors may have the greatest chance of avoiding proofreading and/or cellular surveillance systems. These studies also concluded that the type of spontaneous alterations, the mutant frequencies and location of changes, completely changed with exposure to overt DNA damage. These and other studies have led to the understanding that mutation processes that result from spontaneous mutagenesis are fundamentally different from those that result from induced mutagenesis (Miller, 1992; Friedberg *et al*., 1995; see below). Because *lacI* mutagenesis has been extensively used, an impressive mutational database has been developed for this target. To take advantage of this, *lacI* has also been employed as a mutagenic target for eukaryotes studies (see below).

Reversion mutagenesis assays typically involve the reversal of a point mutation to restore a selectable function and have been used extensively in bacterial and fungal systems. Strains carrying a mutation in a gene that blocks a step in an important process, such as the histidine biosynthetic pathway (i.e. His$^-$), cannot grow on minimal media lacking histidine. Since only cells which can synthesize histidine (His$^+$) can grow on this medium, it becomes possible to directly select for even rare His$^+$ revertant cells (e. g. 10^{-4}–10^{-7}). The Ames test is an application of a His$^-$ to His$^+$ reversion assay in the bacterium *Salmonella typhimurium* (Ames *et al*., 1975). The Ames genotoxicity test involves exposing the appropriate His$^-$ tester strain to some candidate mutagenic compound, plating on minimal medium and then determining if exposure resulted in an increased frequency of His$^+$ revertant colonies. In an effort to apply this bacterial assay to genotoxicity in mammalian systems, a compound is first incubated with rat liver extracts, which simulates the metabolism of a potential mutagenic agent occurring in a whole animal. Due to the fact that most agents known to cause cancer (carcinogens) are also mutagens, this assay led to an extensive survey of chemical compounds (Friedberg *et al*., 1995). The numbers of natural and synthetic chemicals that have been identified as mutagens by the Ames test is impressive and suggested that the human repair processes must cope with substantial exposure to mutagens (Sancar, 1995). Although most of the mutagens identified by the Ames test were also mutagenic when tested in mammalian systems, some substances exhibited different mutagenic potential in prokaryotes and in animals (Friedberg *et al*., 1995; Siedman *et al*., 1985). These and other observations underscored the importance of developing convenient mutagenesis assays in mammals to provide better genotoxicity estimates for humans.

Analysis of Mutagenesis in Mammalian cells

Factors such as genome size, genome complexity, a paucity of convenient genetic selection strategies (as compared to bacteria and fungi) and long generation times have presented a special challenge to molecular analyses of mammalian mutagenesis. To minimize some of these problems, much of the early molecular mutagenesis studies were conducted on target genes carried on small vectors derived from *Simian virus 40* (SV40) (Siedman *et al*., 1985). The general strategy was to infect a susceptible mammalian cell line, then after replication in these cells to recover the vector DNA and examine for the presence of mutations. The development of 'shuttle vectors' that can be propagated in both mammalian and bacterial hosts substantially enhanced the utility of these systems (**Figure 2**). These vectors usually consisted of a prokaryotic mutagenic target, SV40 sequences for replication in mammalian hosts, an origin of replication for bacteria and an antibiotic selection gene (Siedman *et al*., 1985; Friedberg *et al*., 1995). The use of established prokaryotic target genes (e.g. *lacI*) has afforded significant advantages in studying mammalian mutagenesis: first, utilization of convenient mutant detection assays in bacteria; second, access to rapid molecular biology methods; and third, ability to compare sequence changes produced in mammalian cells with the extensive mutagenic database built up over decades of bacterial mutagenesis research (Siedman *et al*., 1985; Miller 1992; Friedberg *et al*., 1995).

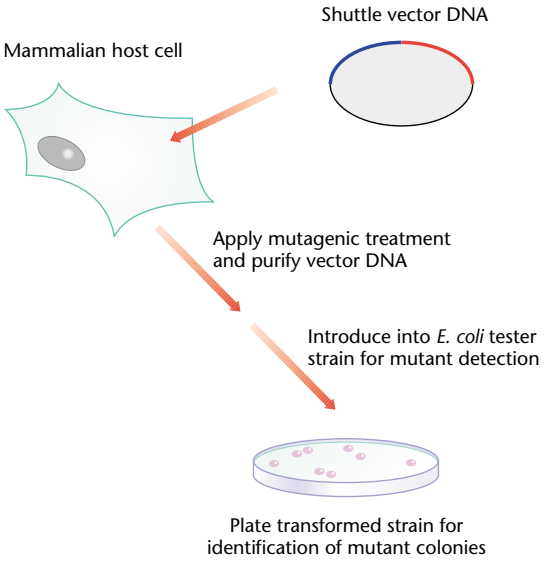

Figure 2 Shuttle vector system. A hypothetical representation of a shuttle vector plasmid map and general approach used to detect mutations that arise in mammalian cells. Shuttle vectors can comprise of several segments, including a mutagenic target sequence (red), sequences for selection and replication in *Escherichia coli* (blue) and sequence for replication in a mammalian cell line. The vector can be introduced into the appropriate mammalian host and then exposed to the mutagenic treatment of interest (e.g. ultraviolet). The vector DNA is retrieved from a cell line, then introduced into the appropriate *E. coli* strain ('tester strain') for rapid detection of mutations in the bacterial target sequence (e.g. *lacI*).

Early studies with shuttle vectors detected astonishingly high spontaneous mutation frequencies which led to some reservations about their reliability. Modifications of shuttle vectors and the identification of alternate cell lines reduced the spontaneous mutant frequencies. However, reservations about the reliability of many shuttle systems persisted, since mutagenesis levels were highly variable and because some viral vectors do not replicate in synchrony with normal cell cycle regulation (Friedberg *et al*., 1995; Sancar, 1995). To better simulate human risk, it would clearly be preferable to examine mutagenesis of a chromosomal target from whole animals, reflecting the normal metabolism of mutagens by the detoxifying enzymes of the liver and normal synchrony of DNA replication. In addition, other issues could be addressed, such as the mutagenic impact of a given chemical carcinogen on various tissue types (e.g. liver, skin or brain).

An advancement that facilitated animal mutagenesis studies was the development of inbred 'transgenic' animals carrying different shuttle vectors. Rodents were artificially constructed that carried a prokaryotic target gene (e.g. *lacI*) that can be retrieved and examined for mutations (Kohler *et al*., 1991). These transgenic animals carry a shuttle vector derived from a prokaryotic virus (λ) incorporated into the genome, which is passively replicated with the surrounding chromosomal sequences during the appropriate phase of the cell cycle (S phase). Following treatment by a mutagen, DNA is isolated from tissues and the vector carrying the mutagenic target is retrieved. The vector can be selectively recovered from the surrounding mouse sequences by subjecting the DNA to a λ *in vitro* packaging extract. The λ packaging produces intact viral particles that, in turn, are used to infect the appropriate *E. coli* tester strain in which mutations are detected. Using these established and convenient molecular genetic methods, mutant frequencies and the nucleotide sequence changes can be readily determined and compared to an extensive mutational database (Kohler *et al*., 1991; Miller, 1992). The availability of these transgenic mutagenesis systems offers a significant improvement in estimating the mutagenic potential of a given treatment. None the less, extrapolating risk to people from animal data can be uncertain and complex. Further studies and the application of rapid technologies such as polymerase chain reaction (PCR) on human tissues will probably play important future roles in enhancing our understanding of mutagenesis in humans (Friedberg *et al*., 1995).

Molecular Basis of Mutagenesis in *E. coli*

It has been demonstrated that *E. coli* and other bacteria have a remarkably accurate combination of replicative and repair processes (an error is produced once every 10^{10} bases replicated). Cells exposed to a variety of mutagens, especially those that produce bulky adducts, result in a dramatic increase in the mutation rate (Weigle, 1953; Friedberg *et al*., 1995; Frank *et al*., 1996). This increase usually does not occur passively, but rather requires the induction of highly specialized damage-processing proteins. The increase in mutagenesis is induced as a part of the global SOS response that is regulated by a repressor (LexA) and RecA protein, which cleaves LexA following damage (Friedberg *et al*., 1995; Snyder and Champness, 1997). Induction of the SOS genes results in an increase of repair activities, an increase in recombination proteins and higher rates of mutagenesis. Following damage, bacteria first attempt to repair DNA in an error-free manner. However, situations can arise where error-free repair is not achieved and it is thought that under these conditions, error-prone replication acts as a last-ditch effort at survival. Two SOS genes called *umuC* and *umuD* were first implicated in this process because inactivation of either gene resulted in total block of SOS mutagenesis. Studies showed that RecA cleaved UmuD protein to a mutagenically active form (UmuD′) and that the UmuCD′ proteins form a complex with RecA and DNA pol III (Frank *et al*., 1996). This complex, referred to as a 'mutasome', is thought to continue DNA synthesis past cyclobutane dimers or bulky adducts (**Figure 3**). This 'bypass' replication is error-prone, allowing misinsertion of bases opposite the lesion, but it is

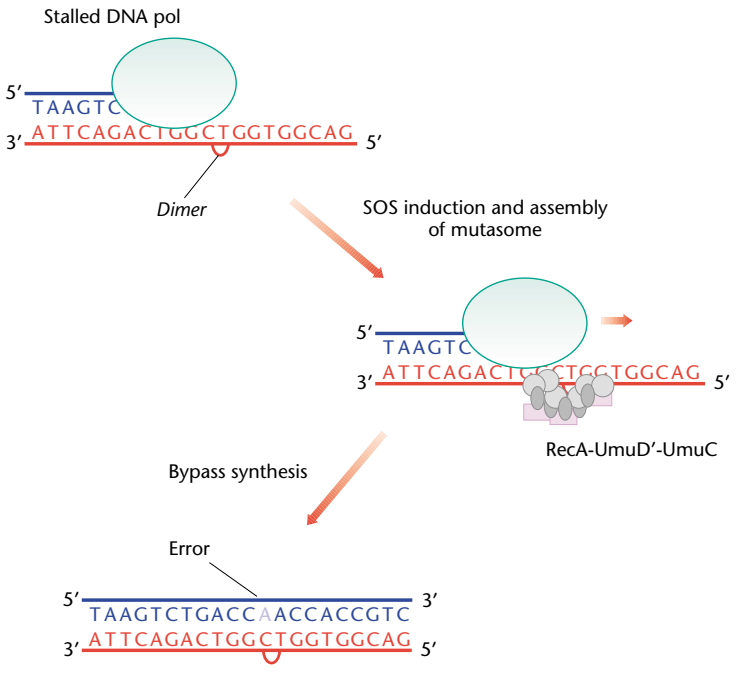

Figure 3 Model for SOS mutagenesis. Replication is blocked when the bacterial DNA pol III encounters and stalls at a bulky lesion such as ultraviolet-induced cyclobutane pyrimidine dimers. Shown here is a C-T dimer. Following the induction of the SOS response the levels of RecA, UmuC and UmuD′ proteins increase and assemble at the lesion. This assembled complex ('mutasome') is thought to direct DNA pol III to synthesize past the lesion, possibly inserting an incorrect base in the nascent strand.

thought to enhance survival by producing intact nascent strands. Error-prone replication is thus referred to as a lesion-tolerance mechanism, employed in emergency conditions (Friedberg *et al*., 1995; Frank *et al*., 1996). The mechanism used by the mutasome to induce DNA pol III to bypass lesions remains the subject of intense biochemical study.

An often overlooked consequence of SOS mutagenesis is that at times of extreme duress there would be a rapid accumulation of mutations and enhanced genetic variation among the survivors. Although the notion of 'inducible evolution' has been controversial, the possibility that inducible mutagenesis might play a role in rapid speciation from populations confronted by extreme selective pressures merits consideration.

Summary

One of the most important activities that an organism undertakes during each round of cell division is the accurate replication of its genome. DNA suffers from spontaneous damage and is bombarded by radiation and chemicals; thus, cells invest a considerable proportion of their resources to repairing damage and keeping mutagenesis to a minimum. DNA necessarily changes as organisms evolve; however, excessive mutagenesis cannot be tolerated by an organism. The study of mutagenesis has led to a better understanding of how cellular mechanisms achieve this balance between genetic integrity and the requirements for change.

Early researchers like the Swiss physicist Jean Weigle, who discovered inducible mutagenesis (Weigle, 1953), could never have foreseen the Pandora's box they opened. The advances since the early days have been spectacular, contributing to fundamental understanding of biological processes, such as SOS repair, error-prone replication and a molecular basis for evolution. These studies will continue to advance our understanding of other complex processes, such as carcinogenesis and how to produce accurate estimates for human mutagenic risk due to exposure to mutagens present in the environment.

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