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REVIEW ARTICLE

Mutagenesis

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ABSTRACT

Mammalian nuclear deoxyribonucleic acid may succumb to more than 60,000 damage episodes per cell per day. If left uncorrected, these adducts, after misreplication past the damaged sites, can give rise to mutations. In nature, the mutations that arise may be beneficial or deleterious, this is the driving force of evolution. An organism may acquire new traits through genetic mutation, but mutation may also result in impaired function of the genes, and in severe cases cause the death of the organism. In the laboratory, however, mutagenesis is a useful technique for generating mutations that allow the functions of genes and gene products to be examined in detail, producing proteins with improved characteristics or novel functions, as well as mutant strains with useful properties.

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INTRODUCTION

Axiological questions related to the nature of mutations, mechanisms for mutagenesis, and the basic components that regulate the rates of mutagenesis have been at the high spot of dramatic study over the past several decades. Investigators in the field of mutagenesis have made tremendous advancements in understanding the origins of mutations and their effects and relevance on broad biological processes, such as carcinogenesis and evolution.

Mutations have a potential for either beneficial or deleterious effects on an organism. To combat the deleterious effects of excessive mutagenesis, sophisticated systems of enzymes have evolved to rectify errors that arise spontaneously in cells or induced following deoxyribonucleic acid (DNA) damage. However, DNA must necessarily change as organisms evolve; thus, some low level of mutagenesis is tolerated and perhaps even promoted to assure normal and healthy levels of genetic variation of populations.[1]

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MUTATION 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 the 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.[1] 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. It is most useful to group mutations based on the DNA sequence alterations, and to limit this discussion to general concepts and classes of mutations.[1]

CLASSIFICATION

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).

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.[1]

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 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.[1]

A base substitution within the protein-coding region of a gene will often result in miscoding of 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. 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).[2]

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. 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.[1,2]

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. 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.[3] Inversions 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 since that segment is 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 with deletions.[4]

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.[4] 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.[3]

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 is 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 is considered to be a driving force for evolutionary change.[1]

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 (DNApol) producing mismatches in the newly synthesized DNA strand (i.e., nascent strand). 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 1,010 replicated bases.[2] 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 DNApol 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. 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 DNApol. 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 by-products 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.[1]

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.[1]

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.[2,5] 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 catalyzes the joining of adjacent pyrimidine bases, and these joined bases, or dimers, usually result in point mutations.[1]

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 CC) but T–T dimers are the most common. Each cyclo but an edimer acts as an obstacle causing a DNApol to stall, although occasionally the DNApol continues synthesis past the dimer and will insert the wrong base. The most prominent class of UV-induced mutations is transitions, followed by other types of point mutations. Stalling by DNApol at UV photoproducts (as well as other related lesions) will induce a cellular DNA repair process. In bacteria, it is called the save our soul (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.[2,6]

Chemical mutagens are categorized into four general groups, based on the mechanism by which they interact with DNA.[3]

1. Base analogs 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, DNApol often stalls at these modified bases. These bulky adducts act as potent inducers of the SOS

response.[2,6] The induction of SOS can lead to the error-prone replication past a range of premutational lesions.

SYSTEMS TO DETECT AND ANALYZE 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.[2,3,7]

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 causing cancer (carcinogens) are also mutagens, this assay led to an extensive survey of chemical compounds.[2] 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.[7] 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.[2,9] These and other observations underscored the importance of developing convenient mutagenesis assays in mammals to provide better genotoxicity estimates for humans.

MOLECULAR BASIS OF MUTAGENESIS

It has been demonstrated that *Escherichia coli* and other bacteria are markably accurate combinations of replicative and repair processes (an error is produced once every 1,010 bases replicated). Cells exposed to a variety of mutagens, especially those that produce bulky adducts, result in a dramatic increase in the mutation rate.[2,3,5,10] 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 pressor (LexA) and RecA protein, which cleaves LexA following damage.[2,3]

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 attempts 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 DNApol III.[6]

This complex, referred to as a "mutasome," is thought to continue DNA synthesis past cyclo but edimers or bulky adducts. This "bypass" replication is error prone, allowing misinsertion of bases opposite the lesion, but it is 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.[2,6]

The mechanism used by the mutasome to induce DNApol III to by-pass 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.[1]

ANALYSIS OF MUTAGENESIS IN MAMMALIAN CELLS

Factors, such as genome size, genome complexity, a paucity of convenient genetic selection strategies (as compared with 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).[9]

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. 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.[2,9]

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 builtup over decades of bacterial mutagenesis research.[2,7,9]

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.[2,8]

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.[9] These transgenic animals carry a shuttle vector derived from a prokaryotic virus (l) 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 an l *in vitro* packaging extract. The l 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 with an extensive mutational database.[7,10] Further studies and the application of rapid technologies, such as polymerase chain reaction on human tissues will probably play important future roles in enhancing our understanding of mutagenesis in humans.[2]

CONCLUSION

One of the most important activities that an organism undertakes during each round of cell division is the accurate replication of its genome. The 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. The 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 Weigle,[11] who discovered inducible mutagenesis, 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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