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MECHANISMS OF SPONTANEOUS MUTAGENESIS: IMPLICATIONS FOR SPONTANEOUS CARCINOGENESIS

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INTRODUCTION

Spontaneous mutations have been defined as mutations that arise by mechanisms that have yet to be identified. While we will discuss the major hypotheses for spontaneous mutagenesis, our main objective is to discuss the roles of DNA damage (especially that caused by normal metabolic reactions) and of DNA repair genes in spontaneous mutagenesis, and the possible relevance of this information to our understanding of spontaneous carcinogenesis.

We have recently published a review on the roles of DNA repair, replication and recombination in spontaneous mutagenesis (1). In the present report, we will only present a brief overview of these topics. Other reviews on spontaneous mutagenesis have appeared (2-8).

WHAT KINDS OF MUTATIONS OCCUR SPONTANEOUSLY?

In a study performed to quantitate the general classes of mutations that occur spontaneously, Hartman et al. (9) classified 83 spontaneous histidine auxotrophs of Salmonella typhimurium and found that 53% were caused by base substitution (either transitions or transversions), 11% were caused by frameshifts (i.e., insertions or deletions of one or a few base pairs), 23% were caused by deletions (i.e., deletions of more than a few base pairs), and 13% were apparently (1) caused by insertions (i.e., insertions of large DNA elements). Since studies of this sort only detect mutations that inactivate a gene product, one can presume that many missense mutations are overlooked in such studies because they have little or no effect on the measured phenotype (i.e., they are silent mutations). Thus, the listed proportion for base substitution mutations is most likely an underestimate, while those listed for the other classes of mutations are most likely overestimates. Therefore, base substitution appears to be the most common form of spontaneous mutation in bacteria. A similar conclusion can also be made for yeast (reviewed in reference 1).

REPLICATION ERRORS

Although the processes of replication, recombination, and repair tend to overlap, this section on the role of replication errors in spontaneous mutagenesis is meant to focus only on the DNA replication that is part of the normal process of cell division. Therefore, damage in a DNA template must not block DNA replication, i.e., it must be miscoding damage rather than noncoding damage. Mechanisms proposed for mutagenesis under these conditions include: (1) base tautomerization, (2) miscoding DNA damage, (3) polymerase errors, and (4) mutators, antimutators and mismatch repair.

Base Tautomerization Based on their model for the structure of DNA, Watson and Crick (10) proposed that transition mutagenesis (A$\leftrightarrow$G, or C$\leftrightarrow$T) may be due to the formation of base tautomers by proton migration. Similarly, Topal and Fresco (11) used tautomerization and base rotation to explain transversion mutagenesis (A or G $\leftrightarrow$ C or T). The essence of this model is that, at the moment of replication, a base in the DNA template develops an inappropriate coding property that leads to the incorporation of an incorrect base in the nascent DNA. Theoretically, base tautomerization could explain most of the spontaneous mutagenesis that is due to base-substitution, however, such a model is inconsistent with the data for the genetic control of spontaneous mutagenesis, which will be discussed below (i.e., the role of DNA repair).

Miscoding Base Damage Modified bases may code differently than unmodified bases. For example, deaminated 5-methylcytosine (i.e., thymine) codes for adenine, and thus will cause a GC $\leftrightarrow$ AT transition. Since there is a good correlation between the occurrence of 5-methylcytosine in the lacI gene and the occurrence of mutation hotspots (12), this suggests that 5-methylcytosine deamination plays a role in spontaneous mutagenesis. Other examples of this type of mutagenesis have been reviewed (1, 8).

Polymerase Errors Loeb and Kunkel (6) have reviewed abundant data showing that DNA polymerases occasionally incorporate incorrect bases. The polymerase error rate is affected by the base sequence of the template, the nature of the misincorporated base, and the nature and source of the DNA polymerase (6). The polymerase error rate is also affected by perturbations in the relative sizes of the pools of nucleoside triphosphates (reviewed in reference 13), and of divalent cations such as Mg^{++} and Mn^{++} (e.g., 14).

While the base sequence of the template clearly has an effect on misincorporation, i.e., base-substitution errors (e.g., 15), it seems also to be an important factor in small and large addition/deletion mutations. In a model for misalignment mutagenesis, Streisinger et al. (16) described how the occurrences of short, redundant base sequences in DNA were correlated with the sites of frameshift mutations and of mutations involving large additions or deletions of DNA. In a related fashion, Ripley (17) and Ripley and Glickman (18) have correlated the occurrence of DNA palindromes with the occurrence of certain frameshift and deletion mutations that are not easily explained by the Streisinger model. Such models of misalignment mutagenesis, and data to support them, have recently been reviewed by Drake et al. (8). These models seem valid because of the good correlation between their predictions and the occurrence of spontaneous addition/deletion mutations at certain chromosomal sites.

Mutators, Antimutators, and Mismatch Repair The mutator and antimutator mutations to be discussed in this section were selected because they are known to affect the normal replication process, or because they do not sensitize cells to killing by DNA damage, or because their mutator effect is independent of the recA gene, i.e., the gene that

controls most mutagenesis after DNA damage induction (reviewed in reference 19). Mismatch repair is generally regarded as a postreplication proof-reading process (20), rather than a repair process for DNA damage.

The isolation and characterization of mutator mutants in such organisms as bacteriophage, bacteria, fungi, Drosophila, and Maize (reviewed in references 5, 21) have been valuable in understanding how spontaneous mutations can occur, but have been much less informative as to how spontaneous mutations do occur. Answers to the latter question come directly from the study and understanding of antimutator mutants. However, only a few antimutator mutants have been found.

Mutations affecting the DNA polymerase of phage T4 result in enhanced transition (22) and transversion (23) mutations. Antimutator phage T4 DNA polymerase mutants have also been described (24, 25). Muzyczka et al. (26) concluded that spontaneous mutagenesis in phage T4 is the result of the antagonistic interaction of the polymerase function (to make replication errors) and the 3'-5' exonuclease function (to correct replication errors).

The possible role in spontaneous mutagenesis of the dnaE mutants of Escherichia coli, which have altered DNA replication polymerases, is probably related to the fidelity of DNA replication. One might consider that the dnaE and lig (DNA ligase) mutants may enhance spontaneous mutagenesis by blocking the replication fork or the resealing of DNA strand breaks, respectively. Both of these actions appear to result in the induction of the SOS phenomenon (reviewed in reference 27), which can lead to enhanced spontaneous mutagenesis, as evidenced by data for the tif (28) and dnaB (29) strains. However, the mutator phenotype of such mutants is recA independent in each case that has been tested, and this recA-independent phenotype has generally been used as a means of defining a replication-error involvement (e.g., 5).

The availability of substrates for DNA synthesis may indirectly affect spontaneous mutagenesis. The purB (purine auxotrophy) mutant (30) would seem to exhibit its antimutator effect by increasing the fidelity of DNA replication via changes in the relative pool sizes of DNA precursors (reviewed in reference 13). DNA precursor pool sizes are also relevant to the regulation of the mutD (31) and tif (32) mutator effects, which suggest that altered deoxynucleoside triphosphate selection can be an important mechanism of spontaneous mutagenesis.

After an incorrect nucleotide has been incorporated into DNA, the cell still can use mismatch repair to correct the error. The mutS, mutH, mutL, and uvrD mutations and the dam mutation are known to affect mismatch repair (33). These mutators are thought to reduce DNA fidelity through a reduction in "proof-reading" function.

Mutator cell lines have also been described in fungi, Drosophila (reviewed in reference 1), and mammalian cells. Meuth et al. (34) described three thymidine auxotrophic lines of Chinese hamster ovary cells that exhibit enhanced spontaneous reversion to thymidine prototrophy, and enhanced spontaneous frequencies of 6-thioguanine and ouabain resistance. Weinberg et al. (35) described three lines of murine T-lymphosarcoma cells that show altered deoxynucleoside triphosphate pools, and enhanced frequencies of spontaneous dexamethasone and 6-thioguanine resistance. Both groups of workers concluded that abnormal deoxycytidine triphosphate pools are responsible for the enhanced rates of spontaneous mutagenesis that they observed.

RECOMBINATION ERRORS

Evidence for a role of genetic recombination in spontaneous mutagenesis has come from an analysis of the meiotic effect in yeast (reviewed in reference 2). That is, certain spontaneous addition/deletion type reversions are much enhanced during meiosis relative to mitosis (36, 37). Because of the strong correlation between addition/deletion reversions and outside marker exchange during meiosis, it was concluded that the reversions were due to unequal crossing-over (36). If spontaneous mutagenesis can result from errors in recombination, then this would explain the meiotic effect in that much more spontaneous recombination occurs during meiosis than during mitosis (e.g., 38). However, such a mechanism only explains a limited amount of spontaneous mutagenesis, since base-substitution reversions and other addition/deletion type reversions do not seem to be enhanced during meiosis (reviewed in reference 7). One way to resolve this question is to compare the genetic control of recombination with that for spontaneous mutagenesis. We have compared the available data on the genetic control of spontaneous recombination and of spontaneous mutagenesis in bacteria (21 genes) and fungi (22 genes) (reviewed in reference 1), and the results indicate that recombination errors do not play a major role in spontaneous mutagenesis. That is, there are many exceptions to the concept that an increase or decrease in the level of recombination should cause a similar change in the level of spontaneous mutagenesis.

REPAIR ERRORS

Bacteria Mutations that sensitize cells to DNA-damaging agents have generally been shown to do so by reducing the cell's capacity for DNA repair. In this section we will evaluate mutations that affect DNA repair for their effects on spontaneous and experimentally-induced mutagenesis. Before doing this, however, it is helpful to understand the concepts of error-free and error-prone DNA repair. The uvrA, uvrB, and uvrC genes, which determine the UV "excinuclease" of E. coli (39), are required for the incision and excision steps in the excision repair of lesions that produce distortions in the structure of DNA, e.g., UV radiation-induced pyrimidine dimers. In general, DNA excision repair is considered to be largely error-free relative to the other major dark-repair process, postreplication repair (40). Thus, compared to wild-type strains, the uvrA, uvrB, and uvrC strains show enhanced mutability after UV irradiation (e.g., 40, 41).

The concept of error-prone repair resulted from the finding by Witkin (42) that a lexA (exrA) strain was not only deficient in the ability to survive UV irradiation, but was also deficient in UV radiation mutagenesis. The lexA gene product is a repressor for the recA gene, a gene required for UV radiation mutagenesis, and the classical lexA mutant is one in which the repressor protein is not removed under conditions that would normally cause derepression (reviewed in reference 43).

From a consideration of the mutations in bacteria and fungi that affect DNA repair (36 and 26 genes evaluated, respectively, as reviewed in reference 1), there is a good correlation in that those mutations that enhance a cell's sensitivity to experimentally-induced mutagenesis also enhance spontaneous mutagenesis, and those mutations that reduce a cell's sensitivity to experimentally-induced mutagenesis also reduce spontaneous mutagenesis. For example, the enhancement of spontaneous mutagenesis in mutants deficient in error-free repair was demonstrated in one study (44) using both uvrA and uvrB strains, and using several base-substitution and frameshift mutation assays. Depending upon the assay used, the spontaneous mutation rate per bacterium per cell division ranged from 1.9- to 6.2-fold

greater for uvrA and uvrB strains than for isogenic wild-type strains. Such data suggest that excisable, cryptic lesions exist in the DNA, and, if not excised, they induce mutations with increased probability.

Mutations that inhibit error-prone repair in UV-irradiated cells also inhibit the enhanced spontaneous mutagenesis seen in uvrB strains. Specifically, uvrB strains carrying lexA, recA, umuC, or the uvrD and recB mutations in combination, have spontaneous mutation rates about 10-fold lower than the uvrB control strains (44). Mutations at recA and lexA reduce the spontaneous mutation rate by about 2-fold in uvr^+ strains (reviewed in reference 44), suggesting that about half of the spontaneous base substitutions in a DNA repair-proficient strain are the result of error-prone repair. The notion that error-prone repair acts on DNA damage, and is not merely affecting replication or recombination, is supported by two pieces of data. (1) Replication-error processes have generally been categorized by their recA independence (reviewed in reference 5), yet, as noted above, about half of spontaneous mutagenesis is recA-dependent. (2) A recombination-error process seems to be ruled out for base-substitution data because the umuC antimutator is recombination proficient (44), and the recombination-deficient recB recF strain shows normal spontaneous mutagenesis (45).

Mutations that enhance error-prone repair, e.g., tif and dnaB, also enhance spontaneous mutagenesis (28, 29).

Similar conclusions concerning the important role that DNA repair plays in spontaneous mutagenesis can be made from the wealth of genetic data that are available for yeast (reviewed in reference 46).

Mammalian Cells Few spontaneous mutagenesis data are available for DNA-damage-sensitive lines of mammalian cells. Liu et al. (47, 48) described a line of Chinese hamster ovary cells that has a mutant form of DNA polymerase. These cells exhibit enhanced UV radiation sensitivity and mutagenesis and enhanced rates of spontaneous mutagenesis, with several forward mutation assays. These cells are not thought to be deficient in excision repair, but only preliminary results are available (49).

One prediction from the conclusion that uvrA and uvrB strains of E. coli are mutators was that cells from individuals with xeroderma pigmentosum (XP) should show a higher rate of spontaneous mutagenesis (44), because such cells are deficient in nucleotide excision repair (reviewed in reference 50). It was also predicted, because of the correlations between mutagenesis and carcinogenesis (e.g., 51), that individuals with XP would show a higher rate of spontaneous carcinogenesis. While the spontaneous mutation rate data for XP cells are not yet available, it is of interest to note that XP individuals have recently been shown to be prone to certain forms of cancer that would not be predicted from their sensitivity to light (52).

SPONTANEOUS DNA DAMAGE

What could be the source of the "spontaneous" mutagenic DNA damage postulated to explain the enhanced spontaneous mutagenesis in DNA repair-deficient strains of bacteria and yeast? One source includes factors present in any mutation assay procedure. The growth rate of the cells, the aeration rate of the culture, the pH and the temperature of the culture medium all have an effect on spontaneous mutagenesis (e.g., 53, 54). The near-UV radiation component of ambient light is known to be mutagenic, either directly (55) or indirectly through its effects on growth media (56). Oxygen apparently induces DNA damage (57) and is mutagenic (reviewed

in reference 58), it causes chromosomal aberrations in Fanconi's anemia cells (59), and has been implicated in spontaneous carcinogenesis (60). Ames (61) has listed numerous mutagens that are present in a wide variety of foods, and these mutagens may also be present in culture media.

Convincing evidence has been presented that spontaneous base-substitution hotspots result from the spontaneous deamination of 5-methylcytosine residues to yield thymine residues, thus causing GC⇔AT transitions (62). Spontaneous depurination (63) and targeted DNA N-glycosylase action (reviewed in reference 64) should induce mutations, because apurinic and apyrimidinic sites tend to result in the insertion of purines (especially adenine) in the nascent DNA (65). The bypass of such lesions requires protein synthesis, i.e., it is an inducible process, and it is associated with mutagenesis, i.e., the bypass process is error-prone (66).

The oxidation of cellular fatty acids could be an important source of "spontaneous" mutagens (reviewed in reference 61). Growth in the presence of phenylalanine, but not the other common amino acids, produces excisable DNA damage that produces mutations in E. coli via error-prone repair (67). Similarly, cysteine (as well as glutathione) is mutagenic in the Ames tester strains if mammalian subcellular preparations are included in the assay (68). As a more general example, a model system exists, using horseradish peroxidase and aromatic pyruvates, for an enzymatic reaction that requires oxygen to produce excited-state molecules (i.e., "UV-like") that can damage DNA (69).

There is also evidence with mammalian cells for the metabolic production of chemical species that damage DNA. Fibroblasts from patients with Bloom's syndrome produce a clastogenic factor that causes chromosomal aberrations in normal human blood lymphocytes, while fibroblasts from normal individuals have no such effect (70). Bloom's syndrome-cells also show enhanced frequencies of spontaneous chromosomal aberrations and sister chromatid exchanges (71), and enhanced spontaneous mutagenesis (72). Such metabolic damage to DNA has also been postulated to explain the characteristics of some other autosomal recessive diseases (73).

In addition, since it appears that DNA can be damaged by normal metabolic reactions such that the damage is recognized by error-prone repair systems (see above), it seems reasonable that damage could also be produced that causes coding errors during replication. For example, although recA strains are nonmutable by X or UV radiation, they are mutable by certain chemicals (e.g., ethyl methanesulfonate and N-methyl-N'-nitro-N-nitrosoguanidine) that produce damage that is presumed to cause miscoding errors during replication (3). Therefore, normal metabolic damage to DNA may also contribute to spontaneous mutagenesis by causing errors in DNA replication.

SUMMARY, CONCLUSIONS AND SPECULATIONS

Spontaneous Mutagenesis There appears to be no dearth of mechanisms to explain spontaneous mutagenesis. For spontaneous base-substitution mutagenesis, data for E. coli and Saccharomyces cerevisiae suggest important roles in spontaneous mutagenesis for the error-prone repair of DNA damage (to produce mutations) and for the error-free repair of DNA damage (to avoid mutagenesis). Data from the very limited number of studies on the subject suggest that about 50% of the spontaneous base substitution mutagenesis in E. coli, and perhaps 90% in S. cerevisiae is due to error-prone DNA repair. On the other hand, spontaneous frameshift and deletion mutagenesis seem to result from mechanisms involving

recombination and replication. Spontaneous insertion mutagenesis has been shown to be important in the strongly polar inactivation of certain loci, but it is less important at other loci.

While most studies have concentrated on mutator mutations, the most conclusive data for the actual source of spontaneous mutations has come from the study of antimutator mutations. Further study in this area, perhaps along with an understanding of the many chemical antimutators and antimutagens, should be invaluable in further clarifying the basis of spontaneous mutagenesis. Perhaps with continued study, the term "spontaneous mutagenesis" will be replaced by more specific terms such as 5-methylcytosine deamination mutagenesis, fatty acid oxidation mutagenesis, phenylalanine mutagenesis, and imprecise-recombination mutagenesis.

Spontaneous Carcinogenesis The idea that metabolically-induced lesions in DNA are important determinants in spontaneous mutagenesis is also of importance in understanding the molecular basis of spontaneous carcinogenesis. Although there is currently much attention being focused upon oncogenes as the ultimate producers of the carcinogenic state, in general, they are "turned on" by a mutation (reviewed in reference 74). The idea that normal metabolism can provide the agents for producing spontaneous mutagenesis provides a mechanism for turning on oncogenes to produce spontaneous carcinogenesis. In support of this concept, the spontaneous activation of a human proto-oncogene has been reported (75).

The concepts in this review on the origins of spontaneous mutagenesis and spontaneous carcinogenesis are diagramed in Figure 1 and described below. Normal metabolic reactions produce products that can damage DNA. The genetic requirements for the repair of this damaged DNA in E. coli suggest that metabolically-produced DNA damage is "UV-like" and not "X-ray-like", and presumably arises through the action of excited-state molecules. This type of damage can presumably be prevented by detoxification, e.g., by the quenching of these metabolically-produced excited states by molecules other than DNA. DNA is also damaged by metabolic stress (e.g., depurination). Depending on the nature of the damage and the repair capacity of the cell, three things can happen: (1) the damage is repaired in an error-free manner and no mutations are produced, (2) the damage is repaired by an error-prone process that can produce a mutation, and (3) the damage changes the coding properties of a base such that normal replication past this altered base produces a mutation. Since these mutations arise without any treatment being applied to the cells, they are called spontaneous mutations. Spontaneous mutations can also arise via replication errors (i.e., the DNA template is undamaged). If a spontaneous mutation occurs in an appropriate gene, then cells can become transformed, and unless the immune system removes these transformed cells, spontaneous carcinogenesis can be the end product of this series of metabolic events.

Figure 1 also demonstrates that there are many mechanisms to explain a person's predisposition to spontaneous carcinogenesis. A person could be a genetic overproducer of products that damage DNA, or be an underproducer of the agents that can detoxify these products. They could be deficient in error-free repair, possess DNA replication enzymes that are error-prone, or be immunologically deficient.

If this model, which is well supported by the bacterial and yeast data for spontaneous mutagenesis, can be confirmed in mammalian cells, then one way of reducing the level of spontaneous carcinogenesis is to identify those enzymatic reactions that modify essential metabolites such that they produce damage to DNA, and then search for nontoxic molecules that can detoxify these reactions, and thus reduce the amount of damage produced in DNA.

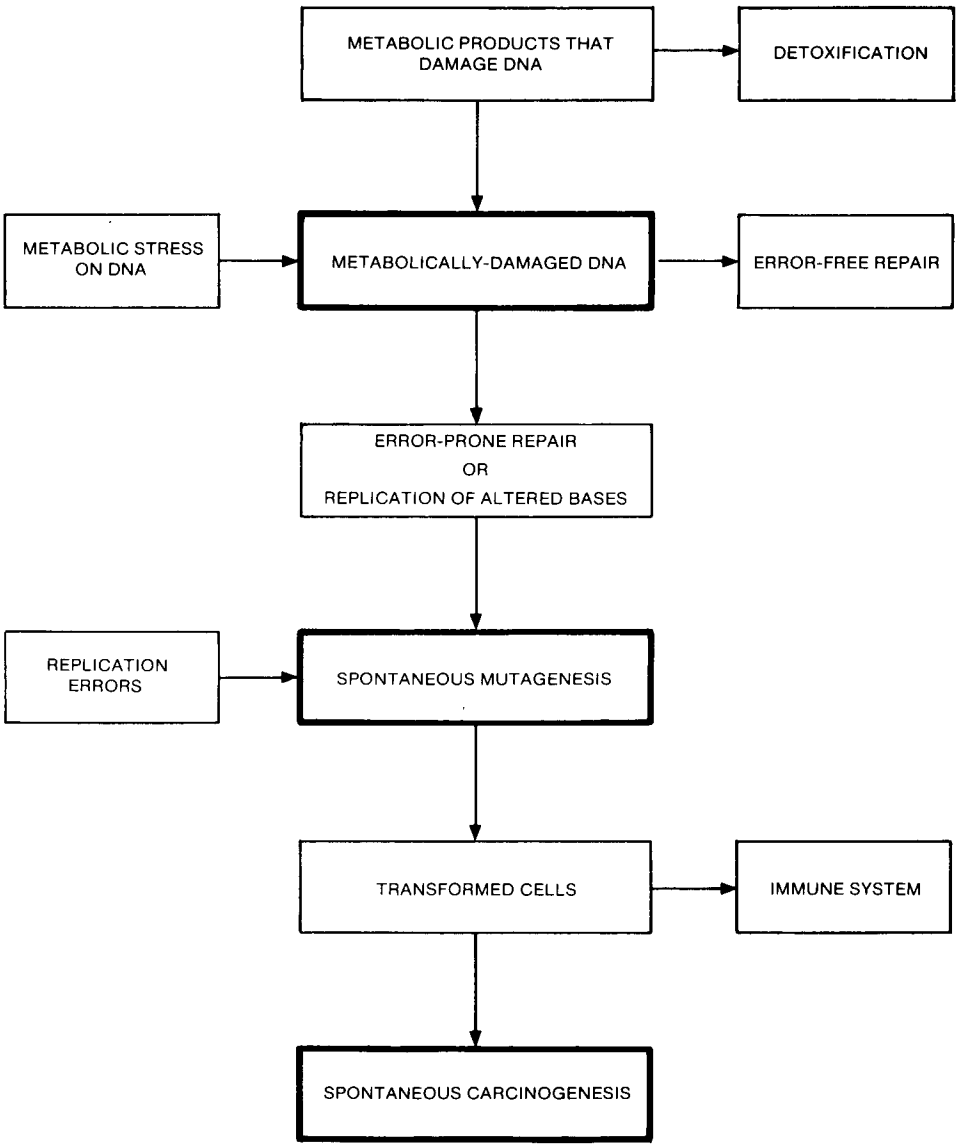

Fig. 1. Model for spontaneous carcinogenesis via spontaneous mutagenesis. See text for discussion.

The world has gone through the phase of identifying environmental carcinogens, and is in the process of cleaning up the environment. The next phase should be to identify the enzymatic reactions that can produce carcinogens from essential metabolites, and begin to clean up or at least detoxify our internal milieu.

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