Photochemistry and Photobiology Vol. 42, No. 6, pp. 801 – 803, 1985
Printed in Great Britain.

0031-8655/85 $03.00+0.00

YEARLY REVIEW

METABOLICALLY-PRODUCED 'UV-LIKE' DNA DAMAGE AND ITS ROLE IN SPONTANEOUS MUTAGENESIS

Introduction

Much of spontaneous mutagenesis in *Escherichia coli* is due to the error-prone repair of DNA damage; such damage is presumably produced in DNA as a natural consequence of normal metabolism and, from knowledge of the genetic control of the DNA repair processes that affect spontaneous mutagenesis, it has been concluded that part of this spontaneous DNA damage is 'UV-like' (Sargentini and Smith, 1981). This conclusion is based in part upon the fact that a deficiency in error-free excision repair, which enhances the spontaneous mutation rate of cells, also renders cells more mutable by UV irradiation, but has little effect on X radiation mutagenesis (reviewed in Sargentini and Smith, 1981). Thus, the repair of this spontaneous DNA damage utilizes the same processes that are required for the repair of DNA damage produced by 254 nm radiation.

A tabulation and review of the available data relevant to the genetic control of spontaneous mutagenesis in several species has recently been published (Sargentini and Smith, 1985). The conclusions from this review concerning the relative roles of DNA replication, recombination and repair in spontaneous mutagenesis will be summarized here, and papers relevant to the 'UV-like' nature of spontaneous DNA damage that leads to spontaneous mutagenesis will be reviewed. Finally, the role of spontaneous mutagenesis in spontaneous carcinogenesis will be discussed.

Where the spectrum of spontaneous mutations has been determined in a single system, base-substitution mutations were found to predominate, and base-substitution assays have been used most frequently for studying the roles of DNA repair genes in mutagenesis (reviewed in Sargentini and Smith, 1985). Therefore, the conclusions reported here are based largely upon data obtained for base-substitution mutagenesis.

How are spontaneous mutations produced?

The three activities that appear to have a major impact on the fidelity of DNA are replication, recombination and repair. Each of these processes has been evaluated as to their role in spontaneous mutagenesis.

DNA replication errors. Several different ways have been described in which spontaneous mutations can arise through errors during DNA replication (e.g. base tautomerization, polymerase errors, abnormal nucleotide pool sizes, palindromes, mismatch repair, etc.) (reviewed in Sargentini and Smith, 1985). However, in the bacterial system, where more data are available, only about 50% of spontaneous mutagenesis can be accounted for by errors in DNA replication. This is based upon the observation that *recA* mutants of *E. coli*, which are deficient in error-prone DNA repair and are nonmutable by UV radiation (reviewed in Witkin, 1976), still show about 50% of the spontaneous mutation rate of wild-type cells (reviewed in Sargentini and Smith, 1985). In general, this *recA*-independent phenotype has been considered as diagnostic for the involvement of replication errors in spontaneous mutagenesis (e.g. Cox, 1976).

Although *recA* strains of *E. coli* are nonmutable by X or UV radiation, they are mutable by certain chemicals that produce lesions in DNA that cause miscoding errors during replication (Kondo *et al.*, 1970). Therefore, it seems reasonable that metabolic reactions could also produce miscoding lesions in DNA, which would produce mutations during normal DNA replication.

Recombination errors. After reviewing the available data for bacteria and fungi on the genetic control of spontaneous mutagenesis and of recombination to see if mutations that increase or decrease recombination have a similar effect on spontaneous mutagenesis, the conclusion was that recombination errors do not play a major role in spontaneous mutagenesis in bacteria or in fungi (Sargentini and Smith, 1985).

DNA repair errors. The conclusion that the error-prone repair of metabolically-damaged DNA is important in spontaneous mutagenesis comes from observations on *E. coli* that mutations that affect DNA repair functions and reduce UV radiation mutagenesis (e.g. *uvrD*, *recB*) have similar effects on spontaneous mutagenesis. Similarly, mutations that block error-free excision repair and enhance UV radiation mutagenesis (e.g. *uvrA*, *uvrB*) also enhance spontaneous mutagenesis, but have little or no effect on X radiation mutagenesis (Sargentini and Smith, 1981).

Support for the importance of DNA repair in spontaneous mutagenesis not only comes from data on bacterial cells, but also from data on fungal and mammalian cells. In the bacterial system, where more data are available, about half of spontaneous mutagenesis has been shown to be due to the error-prone repair of damaged DNA, and about half is presumed to be due to errors made during DNA replication (reviewed in Sargentini and Smith, 1985).

Spontaneous DNA damage that is 'UV-like'

Although a part of the spontaneous DNA damage that leads to spontaneous mutations has been inferred to be 'UV-like', based upon a consideration of the types of DNA repair systems that affect spontaneous mutagenesis, the mechanism(s) by which this 'UV-like' damage is produced in DNA is not known. However, a model system exists, using horseradish peroxidase and several different substrates, for an enzymatic reaction that requires oxygen and produces excited-state molecules (i.e. 'UV-like') that can damage DNA (Cilento, 1980) and proteins (Rivas *et al.*, 1984). Furthermore, metabolizing cells exhibit low levels of chemiluminescence (Cadenas, 1984; Quickenden *et al.*, 1985), which is diagnostic for the presence of excited-state molecules. Excited-state species have also been observed as a consequence of lipid peroxidation in microsomes (Cadenas *et al.*, 1984).

One can predict, therefore, that one possible way to reduce the level of spontaneous mutagenesis would be to introduce molecules into cells that can quench excited states, and therefore prevent the formation of 'UV-like' damage in DNA. Photochemists commonly use quenchers of excited states in their research, but these quenchers are generally toxic to cells. Quenchers will have to be found that are nontoxic and freely enter cells before they can be used in studies on spontaneous mutagenesis (see below).

A recent paper on mutagenesis in V79-4 Chinese hamster cells suggests that the types of mutants produced in the HGPRT locus are different for spontaneous and ionizing radiation mutagenesis (Brown and Thacker, 1984). Although these data don't allow the conclusion that the spontaneous DNA damage in mammalian cells is 'UV-like', they at least suggest that it is *not* 'X-ray-like'.

Thymine residues in DNA are very susceptible to damage by oxidizing agents and ionizing radiation, and the major detectable product is thymine glycol. However, thymine products of the 5,6-dihydroxydihydrothymine type are also formed in the DNA of HeLa cells by exposure to either far-UV or near-UV radiation (Hariharan and Cerutti, 1977). One of the activities of endonuclease III in *E. coli* is its thymine glycol-DNA glycosylase activity. Surprisingly, a *nth* mutant (deficient in endonuclease III) is not sensitive to killing by H_2O_2 or ionizing radiation, nor is it unusually susceptible to the mutagenic effects of 'oxidizing agents'; however, it demonstrates a 4- to 22-fold enhancement in spontaneous mutagenesis (Arg^+ reversion) (Cunningham and Weiss, 1985). It would be of interest to compare the UV (near and far) and X-ray mutabilities of this strain, in the hope that these data may help to identify the chemical nature of the spontaneous DNA damage that leads to spontaneous mutagenesis, i.e. is it 'UV-like' or 'X-ray-like'.

Antimutagens

An extensive review on antimutagens has been published (Clarke and Shankel, 1975). More recent papers will be reported here.

Selenium not only reduces the spontaneous mutation level in *Salmonella typhimurium*, but also reduces the mutagenicity of several chemicals (Martin and Schillaci, 1984). Cobalt(II) ions have also been shown to be antimutagenic for chemical mutagenesis (e.g. Mochizuki and Kada, 1982), and for spontaneous mutagenesis in a *Bacillus subtilis* mutator strain having an altered DNA polymerase III (Inoue *et al.*, 1981). More recently, germanium oxide has been found to be antimutagenic toward chemically-induced frameshift mutations (Kada *et al.*, 1984).

In view of the evidence (discussed above) that spontaneous mutagenesis is associated with the metabolic production of 'UV-like' lesions in DNA, it would be of interest to determine if certain antimutagens, such as selenium and cobalt(II), are capable of quenching the excited states of biological molecules.

Spontaneous carcinogenesis

The importance of spontaneous mutagenesis to spontaneous carcinogenesis has been discussed (Sargentini and Smith, 1981; Smith and Sargentini, 1985). For example, since excision repair-deficient bacteria show enhanced spontaneous mutagenesis, and because of the correlations between mutagenesis and carcinogenesis (e.g. McCann *et al.*, 1975), it has been predicted that patients with the heritable disease xeroderma pigmentosum (XP), which renders their cells deficient in excision repair, should show an abnormal amount and/or types of internal organ cancers (Sargentini and Smith, 1981). It is of interest to note, therefore, that, based upon evidence from normal individuals and from individuals with XP, a proficiency in DNA repair does protect against internal neoplasia (Kraemer *et al.*, 1984).

A deficiency in DNA repair, however, may not be the only mechanism for predisposing a person to spontaneous carcinogenesis. For example, a person could be a genetic overproducer of products that damage DNA, or be an underproducer of agents that can detoxify (e.g. quench) these products. A person could be deficient in error-free repair, possess DNA replication enzymes that are error-prone, or be immunologically deficient. A genetic abnormality in any of these processes would be expected to predis-

pose a person to spontaneous carcinogenesis (Smith and Sargentini, 1985).

KENDRIC C. SMITH
NEIL J. SARGENTINI

Department of Radiology
Stanford University School of Medicine
Stanford, CA 94305, USA

REFERENCES

Brown, R. and J. Thacker (1984) The nature of mutants induced by ionising radiation in cultured hamster cells. I. Isolation and initial characterisation of spontaneous, ionising radiation-induced, and ethyl methanesulphonate-induced mutants resistant to 6-thioguanine. *Mutat. Res.* **129**, 269–281.

Cadenas, E. (1984) Biological chemiluminescence. *Photochem. Photobiol.* **40**, 823–830.

Cadenas, E., H. Sies, A. Campa and G. Cilento (1984) Electronically excited states in microsomal membranes: use of chlorophyll-*a* as an indicator of triplet carbonyls. *Photochem. Photobiol.* **40**, 661–666.

Cilento, G. (1980) Photochemistry in the dark. *Photochem. Photobiol. Rev.* **5**, 199–228.

Clarke, C. H. and D. M. Shankel (1975) Antimutagenesis in microbial systems. *Bacteriol. Rev.* **39**, 33–53.

Cox, E. C. (1976) Bacterial mutator genes and the control of spontaneous mutation. *Annu. Rev. Genet.* **10**, 135–156.

Cunningham, R. P. and B. Weiss (1985) Endonuclease III (*nth*) mutants of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **82**, 474–478.

Hariharan, P. V. and P. A. Cerutti (1977) Formation of products of the 5,6-dihydroxydihydrothymine type by ultraviolet light in HeLa cells. *Biochemistry* **16**, 2791–2795.

Inoue, T., Y. Ohta, Y. Sadaie and T. Kada (1981) Effect of cobaltous chloride on spontaneous mutation induction in a *Bacillus subtilis* mutator strain. *Mutat. Res.* **91**, 41–45.

Kada, T., H. Mochizuki and K. Miyao (1984) Antimutagenic effects of germanium oxide on Trp-P-2-induced frameshift mutations in *Salmonella typhimurium* TA98 and TA1538. *Mutat. Res.* **125**, 145–151.

Kondo, S., H. Ichikawa, K. Iwo and T. Kato (1970) Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. *Genetics* **66**, 187–217.

Kraemer, K. H., M. M. Lee and J. Scotto (1984) DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. *Carcinogenesis* **5**, 511–514.

Martin, S. E. and M. Schillaci (1984) Inhibitory effects of selenium on mutagenicity. *J. Agric. Food Chem.* **32**, 426–433.

McCann, J., N. E. Springarn, J. Kobori and B. N. Ames (1975) Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. *Proc. Natl. Acad. Sci. USA* **72**, 979–983.

Mochizuki, H. and T. Kada (1982) Antimutagenic action of cobaltous chloride on Trp-P-1-induced mutations in *Salmonella typhimurium* TA98 and TA1538. *Mutat. Res.* **95**, 145–157.

Quickenden, T. I., M. J. Comarmond and R. N. Tilbury (1985) Ultraweak bioluminescence spectra of stationary phase *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Photochem. Photobiol.* **41**, 611–615.

Rivas, E. I., A. Paladini, Jr. and G. Cilento (1984) Photochemical-like destruction of tryptophan in serum albumins induced by enzyme-generated triplet species. *Photochem. Photobiol.* **40**, 565–568.

Sargentini, N. J. and K. C. Smith (1981) Much of spontaneous mutagenesis in *Escherichia coli* is due to error-prone DNA repair: implications for spontaneous carcinogenesis. *Carcinogenesis* **2**, 863–872.

Sargentini, N. J. and K. C. Smith (1985) Spontaneous mutagenesis: the roles of DNA repair, replication, and recombination. *Mutat. Res.* **154**, 1–27.

Smith, K. C. and N. J. Sargentini (1985) Mechanisms of spontaneous mutagenesis: implications for spontaneous carcinogenesis. In *Radiation Carcinogenesis and DNA Alterations* (Edited by F. J. Burns, A. C. Upton and G. Silini). Plenum Press, New York. In press.

Witkin, E. M. (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacterial Rev.* **40**, 869–907.