Inducible Pathways in Deoxyribonucleic Acid Repair, Mutagenesis and Carcinogenesis

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The aim of this short review is to summarize our recent knowledge and current ideas about the genetic control and molecular mechanisms of mutagenesis in bacteria, in order to discuss possible mechanisms of malignant transformation in mammalian cells.

This review is neither a good source of relevant references nor a critical evaluation of quoted work; these can be found elsewhere (Radman, 1975, 1976; Witkin, 1976; Radman et al., 1977). It is hoped that by studying mutagenesis in bacteria, we may learn something about early steps in the malignant transformation of mammalian cells (Radman, 1976; Radman et al., 1977), the principal arguments being as follows. (1) Not only can bacterial mutagenesis tests efficiently discriminate between carcinogenic and non-carcinogenic chemicals (McCann et al., 1975), but there exists a quantitative correlation between the mutagenic potency in Salmonella tester strains and carcinogenic potency in animals for a heterogeneous group of chemical carcinogens. The respective potencies of these compounds range over five orders of magnitude (Meselson & Russell, 1977). (2) If we accept the hypothesis that carcinogenesis in animals is caused by somatic mutagenesis, and if we trust Meselson & Russell’s (1977) animal carcinogenicity–Salmonella mutagenicity correlation, then it appears likely that the basic mechanisms of mutagenesis in both bacteria and animal somatic cells (by these chemical carcinogens) must be very similar. There is growing evidence that somatic mutagenesis is the basis of cancer in man (see Cairns, 1975).

Recent studies with cell cultures support this conclusion: the comparison between transformation and mutation frequencies in the same benzo[a]pyrene-treated primary cell culture shows that transformation could be provoked by mutation at a single hypermutable locus (see Huberman et al., 1976). Analogously to forward and backward mutagenesis, the same carcinogen can provoke both induction of transformed-cell clones and their reversion to normal-cell clones, often accompanied by temperature-sensitivity (Bouck & di Majorca, 1976). This is highly suggestive of forward and backward mutagenesis in a gene with a protein product. Inspired by the recent knowledge about pleiotropic gene control in mutagenesis of Escherichia coli (to be summarized below), we have proposed a working model for malignant transformation through mutagenesis of a single locus (Radman et al., 1977; reproduced in Fig. 1) which also includes viral transformation.

Pathways of DNA repair related to mutagenesis in bacteria

Any process at the level of DNA leading to improved survival will be referred to as DNA repair. The best known is the excision–resynthesis repair leading to elimination of DNA lesions in a usually error-free fashion [see Hanawalt & Setlow (1975) for references]. Therefore the more efficient the excision repair, the less efficient the mutagenesis. However, we are particularly interested here in how mutations occur. With regard to mechanisms and genetic control, mutagenesis in E. coli occurs by two major pathways (Radman et al., 1977).

Direct mutagenesis. Direct mutagenesis is provoked by subtle modifications of DNA bases which are incorporated and/or copied by DNA polymerases as if they were another base. Such direct mispairings are not efficiently recognized as such by E. coli DNA polymerases in spite of their nucleotide discrimination (Gillin & Nossal, 1975) and 3’ to S’ exonuclease (proof-reading) activity (Brutlag & Kornberg, 1972). Direct mutagens are, for example, tautomers and isomers of normal bases (see Topal & Fresco, 1976), base analogues, such as 2-aminopurine and 5-bromouracil, deaminating agents (transforming, e.g., cytosine to uracil), alkylating agents producing O6-alkylguanine etc.
(for a review see Drake & Baltz, 1976). However, such replicational errors are subject to another DNA-repair system that it is probably involved in gene conversion: the 'generalized' methylation of adenine residues in E. coli DNA provides for discrimination between parental and newly synthesized strands for an excision–resynthesis type of mismatch correction (M. Radman, R. E. Wagner & M. Meselson, unpublished work). Thus inhibition of DNA methylation is mutagenic. When compared with other mutagens on a molar basis, these direct mutagens appear as only weak mutagens and weak carcinogens (Meselson & Russell, 1977).

Indirect mutagenesis. This mutagenesis is provoked by non-coding DNA lesions which inhibit DNA synthesis. Strongest mutagens and carcinogens are indirect mutagens in bacteria, such as u.v. and ionizing radiation, active forms of aflatoxin B1, acetylamino-fluorene, benz[a]pyrene, mitomycin C etc. U.v.-induced pyrimidine dimers are the best-studied lesions of this type that cannot be copied by the DNA-replication machinery of untreated E. coli either in vivo or in vitro (Villani et al., 1976; Radman et al., 1977). Experiments in vitro, in which the turnover of nucleoside triphosphates to free monophosphates was followed during complete arrest of DNA synthesis by pyrimidine dimers, have revealed that DNA polymerases can incorporate non-complementary bases opposite pyrimidine dimers. However, DNA polymerase is prevented from continuing DNA synthesis across and past pyrimidine dimers by its 3' to 5' exonuclease (proof-reading) activity, which excises 3'-hydroxyl-terminal mismatches and causes idling of the DNA polymerase at each dimer in spite of continuous triphosphate utilization.

This finding is in agreement with the fact that extracellularly u.v.-irradiated bacteriophages λ and φX174 are killed, but not mutagenized, when infecting intact host cells (Weigle, 1953; Bleichrodt & Verheij, 1974). These bacteriophages are mutagenized, even if not irradiated, when infecting u.v.-irradiated host cells after an optimal time delay after irradiation (Defais et al., 1976, and references therein). It appears that indirect mutagens both require and cause induction of a cellular mutagenic DNA repair (SOS repair) which permits DNA synthesis across and past pyrimidine dimers by its 3' to 5' exonuclease (proof-reading) activity, which excises 3'-hydroxyl-terminal mismatches and causes idling of the DNA polymerase at each dimer in spite of continuous triphosphate utilization.

SOS-induction pathway in E. coli

Induction of the mutagenic SOS repair is part of a complex cellular response, a kind of emergency syndrome (SOS induction) provoked by DNA lesions, which also involves the temporary arrest of cell division (filamentous growth), arrest of oxidative metabolism and induction of prophage in lysogenic bacteria (for review see Witkin, 1977). In analogy to bacteriophage λ induction, it was presumed that both filamentous growth (Witkin, 1967) and SOS repair (Radman, 1974) are accomplished through inactivation of some 'bacteriophage λ-like' cellular repressors. Since filamentous growth can be induced by much lower u.v. doses than the SOS repair (Bridges & Green, personal communication), it is conceivable that each pleiotropic expression of SOS induction is under control of its own specific repressor and that these may vary in amount and/or susceptibility to a common inducer. The ultimate inducer for prophage λ is some as yet unidentified proteinase which cleaves prophage-λ repressor in the course of u.v. and mitomycin C induction (Roberts & Roberts, 1975). The fact that some proteinase inhibitors can suppress u.v.-induced mutagenesis, prophage-λ induction and filamentous growth, at non-toxic concentrations, supports the idea of the common proteolytic inducer (Rossman et al., 1977; Radman et al., 1977). What activates the proteolysis of
repressors is not known, although we have suggested that nucleoside monophosphates produced by the proof-reading activity during idling of the DNA polymerases at non-coding DNA lesions may play the role of cofactor (Radman et al., 1977). Elegant work on mutants deficient in SOS induction (recA and lex) and on SOS temperature-inducible mutants (tif, dnaB, ligts), as well as the phenomenology of SOS induction, was exhaustively reviewed by Witkin (1976).

**SOS induction in mammalian cells?**

A number of phenomena in radiobiology and in chemical carcinogenesis, indicative of the existence of (an) inducible DNA-repair system(s) in mammalian cells, have been reviewed (Radman, 1976). Re-activation of irradiated Herpes simplex virus by u.v. and X-ray irradiation of the host cell and its inhibition by caffeine, which is an inhibitor of a particular post-replication repair likely to be involved in fixation of malignant change, is one such example (Hellman et al., 1976). Unfortunately, there are no available data on the mutagenesis of intact and irradiated viral genomes in irradiated cells. Only one experiment has been performed to test for the induction of DNA repair in mammalian cells: the post-replication repair of u.v.-irradiated cells was shown to be significantly improved by pre-irradiation with a small u.v. dose followed by a 5h incubation period (Setlow, 1977).

**A working hypothesis for malignant transformation**

The ideas about indirect mutagenesis could be applied to initiation and promotion in chemical and radiation-induced carcinogenesis (for a review see Ryser, 1971). The questions that cannot be answered as yet are: is initiation a persisting DNA lesion or a mutation and is promotion mutation-fixation or mutation-expression? The simple working hypothesis, shown in Fig. 1, assumes the following: (1) the transformed state is a state of permanent promotion provoked by an inducer proteinase, which may be a plasminogen activator (Wigler & Weinstein, 1976) or some related intracellular proteinase; (2) multiple phenotypic expressions of transformed cells are provoked by induction of whole batteries of genes through proteolytic cleavage of cellular repressors and/or by activation of gene products by proteolytic cleavage; (3) malignancy can be brought about by (single) mutation(s) in the repressor gene (R) of the inducer proteinase; (4) as in bacteria, mutation-fixation requires induction of SOS repair, perhaps by the same inducer proteinase (for the sake of simplicity); (5) maximum mutagenesis results from simultaneous SOS repair, DNA replication and unrepaired DNA lesions; mutations in the R gene can inactivate the repressor of the inducer-proteinase gene and cause stable transformation; (6) promotion is a temporary inactivation of the inducer-proteinase repressor (see the legend to Fig. 1). It is implicit in this model that the malignant character should be suppressible: malignancy should last only as long as inducer proteinase is active and only as long as repressors remain sensitive to it. Transformation provoked by mutations in the R gene should be recessive, i.e. suppressed by a normal cellular genome, only as long as an intact R gene is present in the cell, and furthermore different independently arising tumour cells should not complement each other. Possible exceptions are (presumably rare) operator (O) mutations, which should be dominant. Both predictions of the model are in agreement with the tumour x normal and tumour x tumour cell-hybridization experiments (Wiener et al., 1974; Stanbridge, 1976).

**Viral transformation.** The simplest application of this model to viral transformation is a constitutive production of a viral inducer proteinase, which is functionally analogous to the cellular inducer proteinase and which is coded for by the viral 'transforming gene'. Integration of viral DNA containing the 'transforming gene' into the cellular genome would help its maintenance in the cell. Such transformed character is expected to be dominant in cell-hybridization experiments. Alternatively, the R gene or its product could also be inactivated by the presence of an integrated specific viral DNA sequence,
Transformation is considered as permanent promotion through permanent activation of the gene coding for inducer proteinase. (a) Co-carcinogens or promoters, such as phorbol esters, hormones etc., could interact directly with the inducer-proteinase repressor and inactivate it reversibly. (b) This is presumed to occur in bacteria: low-molecular-weight DNA metabolites, produced as a consequence of DNA lesions, could act as cofactors in the proteolysis of cellular repressors. (c) Cyclic AMP could act as a competitor of low-molecular-weight DNA metabolites (b) thus preventing proteolysis of repressors [see Radman et al. (1977) for arguments]. (d) Inducer proteinase is supposed to cleave (or activate the cleavage of) genetic repressors and/or activation of gene products in the presence of positive effectors (b). Inducer proteinase should not cleave its own repressor, otherwise promotion would become irreversible. Inactivation of a class of cellular repressors would lead to partial de-differentiation and mitotic stimulation as well as de-repression of the mutagenic SOS repair. A low constitutive concentration of inducer proteinase is necessary to account for mutagenesis and carcinogenesis of solitary carcinogens (initiators) in the absence of promoters [weak promotion through (b)]. (e) Mutational changes can inactivate the $R$ gene leading to a permanent de-repression of the inducer-proteinase gene, i.e. to a stable transformation. $R$ gene or its product could also be inactivated by the presence of an integrated specific viral DNA sequence (see the text).
and this could occur either by the inactivation of the \( R \) gene, by integration of the viral DNA into the \( R \) gene, or by activation of the inducer-proteinase gene, by an integration of viral DNA into the operator (\( O \)) region or by the product of viral (transforming) gene integrated somewhere else. In the first case malignant character should be recessive as discussed above, but in the second and third cases malignancy should be dominant. Both recessivity and dominance was observed with virus-transformed cells, but transformation may be dominant and tumorigenesis may be recessive (Wiener et al., 1974; Croce & Koprowski, 1975; Jha & Ozer, 1976). Furthermore the non-complementation between chemically transformed and virus-transformed cells (Wiener et al., 1974) is also consistent with our model. The quoted cell-hybridization experiments are not satisfactory as the test of the proposed model, because none have been done with newly transformed cells issued from the same initial clone.

Finally, and most specifically related to this model, is the positive correlation (1) between the increased temporary production of a proteinase (plasminogen activator) and promotion both \textit{in vivo} and \textit{in vitro} (see Wigler & Weinstein, 1976) and (2) between the permanent increase in plasminogen activator activity and cell transformation by both chemicals and virus (Rifkin et al., 1975).

On the other hand, proteinase inhibitors which inhibit SOS induction in \textit{E. coli} (Meyn et al., 1977; Radman et al., 1977) can delay and decrease induced carcinogenesis in mice (Troll et al., 1975). However, relevant to our proposal is the measurement of intracellular proteinase activities which has not been performed. Evidence is being accumulated that the transition from the G1-phase to the S-phase in cells may be mediated by a trypsin-like proteinase acting intracellularly [see Brown et al. (1977) and references therein].

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Human Genetic Disorders with Defects in the Repair of Deoxyribonucleic Acid

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In the human population there are a number of genetic disorders whose symptoms suggest that the affected individuals are unusually sensitive to radiations or chemical mutagens. The first of these to be studied at the molecular level was the autosomal recessive disorder xeroderma pigmentosum. The skin of xeroderma pigmentosum patients is extremely sensitive to sunlight and they develop a variety of different skin lesions culminating in multiple skin cancers and early death (Robbins et al., 1974).

Cultured cells from most of these individuals are hypersensitive to the lethal effects of u.v. irradiation and are unable to excise u.v.-induced cyclobutane pyrimidine dimers from their DNA (Cleaver & Bootsma, 1975). Genetic studies have revealed the existence of at least five different complementation groups (designated A–E) all partly or totally defective in excision repair after U.V. (Kraemer et al., 1975). A sixth class of patients (termed xeroderma pigmentosum variants) have classical xeroderma pigmentosum symptoms, but their cells show normal or near-normal survival after U.V. irradiation and normal excision repair of pyrimidine dimers (Robbins et al., 1974; Cleaver & Bootsma, 1975; Lehmann et al., 1975). We have shown that fibroblasts from these xeroderma pigmentosum variants have a defect in a different repair process, termed post-replication repair (Lehmann et al., 1975).

The normal DNA-replication machinery is unable to cope with a template containing damaged bases. Growing cells are not, however, able to excise all the damage from their DNA before replication and special mechanisms have been evolved in order to deal with the replication of these damaged bases, so that intact daughter DNA strands can eventually be synthesized. These mechanisms, which are as yet rather poorly understood, are termed post-replication repair (Lehmann, 1974). After u.v. irradiation, it seems that gaps are left in the daughter DNA strands, possibly opposite the damaged bases, and these gaps are subsequently sealed. This is observed as a conversion of low-into high-molecular-weight newly synthesized DNA in u.v.-irradiated cells, as measured