# ADVANCES IN CANCER RESEARCH

GEORGE KLEIN
SIDNEY WEINHOUSE

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# ADVANCES IN CANCER RESEARCH

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# CANCER: SOMATIC-GENETIC CONSIDERATIONS

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## I. Introduction

For some twenty years (Burnet, 1957) I have been a deeply interested onlooker in the field of theories of cancer etiology, always with a prejudice in favor of "somatic mutation" as against "cancer virus" theories. For reasons obvious to those familar with both fields, my point of view has been greatly influenced by what has happened over the same twenty years in immunology. In view of the recent tendency for oncologists to become increasingly aware of genetic and somatic-genetic factors in the etiology of cancer, and the associated wave of interest in mutagenesis and DNA repair, I endeavored to give a semipopular summary of current views of cancer etiology in the 1976 Brailsford Robertson Memorial Lecture at the University of Adelaide, which was subsequently published (Burnet, 1977). The present contribution is essentially an expansion of that address, and it will be convenient to summarize its conclusions as an introduction to the extended version.

It was contended that modern work allowed one to make a series of simple broadly based statements which allowed a useful and, within the limits of present understanding, an approximately true picture of the nature of malignant disease. The theme of the lecture was in the tradition of many earlier discussions of the nature of malignancy, from Cohnheim (1889) onward, and including, in recent years, Markert

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(1968), Potter (1969), Anderson and Coggin (1974), and, with some reservations, Dulbecco (1977). In all their recent discussions cancer is more or less clearly envisaged as a disease of differentiation. In this expansion I have used as a theoretical background the ideas on the genetics of differentiation that have been developed by Davidson, Britten, and their collaborators (Britten and Kohne, 1968; Britten and Davidson, 1969; Davidson *et al.*, 1973; Davidson and Britten, 1974).

The essence of the approach can be summarized as follows:

(A) 1. Carcinogens are mutagens, either directly or after modification by tissue enzymes.

2. Mutation, germ-line or somatic, is the result of alteration from the normal sequence of nucleotides in some relevant segment of DNA, with retention of ability to undergo the normal process of replication.

3. Such "informational" changes in DNA result from chemical damage to nucleotides arising either spontaneously (thermally) or by the action of physical, chemical, or viral mutagens, followed by error-prone DNA repair.

(B) 1. Mammalian DNA includes ±30% of structural DNA coding for specific proteins and ±70% which, among other functions, must control the distribution and timing of gene expression needed for the development and maintenance of the organism. The nonstructural DNA is probably responsible for a wide range of functions, but it can be usefully spoken of as "control DNA."

2. Control DNA is chemically equivalent to structural DNA, is handled by the same enzymes, and is subject to the same types of damage and repair as structural DNA.

3. Most of the germ-line mutations that eventually become relevant to evolutionary change involve control DNA.

(C) 1. Somatic mutation in mammalian cells is always rare (of the order $10^{-6}$ or less) and involves initially a single cell.

2. Except under quite exceptional conditions, the only type of somatic mutation that is experimentally or observationally demonstrable is one in which the mutant cell gives rise to a large clone of descendant cells visible as a discrete anomaly of pigmentation, benign or malignant tumor, or a monoclonal excess of abnormal circulating cells.

3. Appropriate tests in human subjects heterozygous for A and B types of the enzyme G6PD or some equivalent marker show that, with some rare exceptions, all malignant tumors of man are of monoclonal nature.

4. More than one somatic mutation can be expressed in a given cell, and a number of important oncological phenomena probably depend

on such multiplicity. (i) Many, perhaps all, cancers require a sequence of two or more genetic errors before malignancy can be expressed. (ii) In any neoplastically proliferating clone, any "structural" or "control" mutation previously present in the initiating cell will be expressed in the tumor cells, and in the case of a structural gene may be detectable by biochemical or immunological techniques.

(D) 1. The readiness with which cancer is induced by mutagens or arises spontaneously may be strongly influenced by genetic (germline) factors. The degree of this influence varies greatly from one type of tumor to another, but with sufficiently comprehensive study a genetic component would probably be demonstrable for all types of malignancy.

2. The process by which a normal cell is transformed to a potential cancer cell normally involves more than one stage, of which not necessarily all are mutational. Further steps by which the malignant clone achieves full expression may include the action of "cocarcinogens," for which evidence of mutagenicity is not available.

(E) 1. Active proliferation of mammalian cells is specially characteristic of the embryonic period.

2. The presence of embryonic or ectopic antigens in tumors, plus a variety of other evidence, indicates that the commonest type of mutation giving rise to cancer is one in which control DNA is changed so as to induce an erroneous program of activation appropriate to some stage of embryonic development.

(F) 1. Not every mutant cell with the potential for malignant growth gives rise to a cancer. There is evidence for immune surveillance, for the importance of tissue factors (? chalones), and in some childhood tumors of spontaneous maturation to a mature nonproliferating form, as inhibitory factors.

2. A number of tumors are dependent on the presence of an appropriate balance of hormonal stimulation if they are to maintain their neoplastic proliferative quality.

## II. DNA, Control and Structural

All one's thinking about cancer is based on the experimental finding that any cancer cell that is accessible to study can be shown to give rise by the standard process of mitosis to a clone of similar cells with the same functional and morphological properties that differentiate them from normal cells of the same organism. The malignant quality is inherited and there is nothing to invalidate the obvious deduction that the malignancy is determined like any other inherited quality by some

difference in the nucleotide sequence of the cellular DNA. When mutation involves a structural gene coding for a known protein, valid evidence of this can be obtained by a comparison of amino acid sequence determinations in normal and mutant proteins. No such approach is possible in regard to malignant change, and by hypothesis the important mutational changes must be located in DNA other than that which makes up the structural genes. There is almost no direct experimental evidence of the specific nature and function of such "control" DNA in mammalian cells. The classical studies of enzyme synthesis in *E. coli* indicate that control processes are mediated for the most part by regulatory genes producing repressor or other proteins that function by specific union with portion of an "operon" adjacent to the gene whose rate of synthesis is being regulated. In view of the existence of the whole genetically controlled process of embryogenesis and growth in higher forms, it is obvious that their control DNA must have many more functions than is the case in *E. coli*. Some will almost certainly function as structural genes whose products are proteins with control functions within the genome; but, if we are to avoid an impossible situation of infinite regression, other types of control must exist. For the present, it seems ligitimate to use the term control DNA to cover all that DNA in the haploid genome that is not identifiable as coding for specific proteins, and use only operational concepts in discussing it. The likelihood that much of the control is by gene products (RNA or polypeptide) is definitely not excluded.

There will undoubtedly be found in due course a wide variety of functional information in the genome beyond that present in structural genes of classical type. Any consideration of the requirements of embryonic development, the features of malignant growth, and of individual differences within the species points however to the most important function being to determine the timing and distribution of phases of activation and repression in structural genes and in other control genes. At a rather unsophisticated level, one can say that each phase in the processes of embryogenesis must require the initiation of a complex sequence of gene activations appropriately coordinated. Once the phase has been completed, the active genes will be repressed or a process of positive activation inhibited by some signal from cells that have reached the required level of development. One must in fact picture a process of information handling analogous to that of a computer-controlled battery of machine tools automatically producing accurately formed metal articles or components.

The extreme morphological complexity of any vertebrate offers *prima facie* evidence for the existence of control DNA of this type.

Even more cogent is the existence of large numbers of individual differences in morphology within a single species or between two closely related species in contrast to the small number of biochemical differences. According to King and Wilson (1975), man and chimpanzee are "sibling species" with almost identical proteins, despite their gross anatomical differences in every detail. Morphological differences in the virtual absence of biochemical or antigenic ones can best be interpreted as resulting from differences in the timing of sequences of gene activation and repression.

Such an interpretation would be in line with the standard analysis of the operon in *E. coli* genetics by Jacob and Monod (1961) and their successors. It is probably immaterial to argument at the operational level how the control DNA is located in the genome, but, following Davidson and Britten (1974), I shall assume that segments are intercalated in tandem among structural genes. It will be accepted, in the absence of any evidence to the contrary, that all control DNA has the normal polynucleotide structure of DNA and is subject to the same types of damage by physical and chemical agents, replicates and is repaired by the same battery of enzymes, and is subject to the same types of informational error as structural DNA.

Experimental evidence in support of this opinion is meager. Lieberman and Poirier (1974) showed equivalent degrees of repair in satellite and main-band DNA in cultured mouse cells. They concluded that repair enzymes had access to damaged satellite DNA, even in heterochromatin regions, and that preservation of sequence fidelity is as important there as in structural DNA.

## III. Mutagenesis and Carcinogenesis

The likelihood that chemical carcinogens act by inducing mutations in the affected somatic cells has been recognized for many years and appears to have been virtually established in recent years by the work from Ames' laboratory (Ames *et al.*, 1973; McCann and Ames, 1976). The important new development has been to show that many carcinogens not demonstrably mutagenic on sensitive bacterial indicator strains give rise to mutagenic derivatives when exposed to the enzymes in crude extracts of mammalian tissue. In the opinion of Ames *et al.* (1972), a considerable number of carcinogens act as frameshift mutagens.

The most important physical carcinogens responsible for experimental and clinical malignancy are ultraviolet light and X-irradiation, both of which are classical mutagens. It follows that most oncologists

now accept as axiomatic that the process by which a cell initiates a malignant clone is an expression of somatic mutation. This is subject to the implicit qualification that the genome of the affected cell may often have been rendered susceptible to the carcinogen in at least three ways: (1) by the presence of germ-line genetic information in the somatic cell genome; (2) by the genetically controlled character of the cellular environment, particularly in regard to levels of general and local hormones; (3) by the presence of one or more prior somatic mutational changes in the cell or its precursors in the cell line.

Despite the many deviations from the normal that have been studied in the biochemical or antigenic qualities of cancer cells, no one has seriously claimed that any key change in a structural gene and its protein product is responsible for the malignant quality. This adds another reason for locating the essential mutation in the control DNA.

Basic study of the mutational process has been a major activity of microbial geneticists in the last two decades; most of the work has been done with *E. coli* and mutagens such as ultraviolet light and a range of chemical agents. In general, the enzymes handling mammalian DNA are equivalent to those active in *E. coli*, and the various repair processes seem to be very similar. One can undoubtedly expect differences corresponding to the much more complex mammalian genome, but the reactions in *E. coli* have already provided information that is highly relevant to important areas of cancer research. Study of the effect of exposure to ultraviolet light on the DNA of *E. coli* has been of special significance.

Long, continued exposure to the ultraviolet component of sunlight is a major cause of human skin cancer; basal cell carcinoma, squamous epithelioma, and malignant melanoma are produced in that order of frequency. Persons with the genetic disease xeroderma pigmentosum show a greatly increased susceptibility to both the inflammatory and the carcinogenic effects of sunlight (Robbins *et al.*, 1974). The pathogenesis of the condition has been intensively studied and shown to be based on inefficiency of the DNA repair process. Cultures of skin fibroblasts from such patients have allowed experiments similar in conception to those used in bacterial genetics, and the results have greatly strengthened the relevance of the *E. coli* model.

The general opinion is that mutation in *E. coli* nearly always results from misrepair of lesions produced by the mutagen. Minimal lesions are usually repaired by the standard constitutive enzyme complex without error, but when DNA damage is more extensive and no adequately intact strands are available to serve as templates, a new polymerase is induced (Sedgwick, 1975). This is a more highly

mutagenic system perhaps because the induced polymerase permits the insertion of noncomplementary nucleotides opposite DNA lesions or deletions.

In reviewing ultraviolet-induced mutation and DNA repair, Witkin (1976) concludes that ultraviolet mutations in *E. coli* are caused by inaccurate repair of affected DNA. Most of the damage by pyrimidine dimer formation is repaired by relatively error-proof mechanisms: photoreactivation, short-patch excision repair, or the major pathways of recombinational postreplication repair. Some other kinds of damage require and induce an error-prone process ("SOS") when single-strand gaps are not reparable by any accurate process. This also results in the derepression of other functions of the bacterium, including prophage induction, cell division delay, and aberrant reinitiation of DNA synthesis. Sedgwick (1975) showed that protein synthesis was required in such repairs and that the protein was involved in some part of postreplication repair and is responsible for induced mutagenesis. Mount (1977) obtained a mutant showing constitutive expression of both error-prone repair and prophage induction.

Evidence that a similar induced error-prone DNA polymerase is concerned in the repair of mammalian cells and in the induction of cancer has been obtained by the use of caffeine, which appears to be a specific inhibitor of the error-prone mechanism in *E. coli*. Maher *et al.* (1975) find a synergistic effect of caffeine on the cytotoxicity of ultraviolet irradiation and of hydrocarbon epoxides on xeroderma pigmentosum cells. More directly, Latarjet (see Dulbecco, 1977) found that the induction of skin cancer in mice by irradiation is strongly inhibited by caffeine, while reductone, which inhibits the error-proof mechanism, is without effect.

It is uncertain how far spontaneous DNA changes lead to error in the nucleotide sequence in the course of their repair. A recent paper by Lindahl (1977) reviews the various types of spontaneous lesions in DNA, of which the commonest is depurination; considerably less frequent are depyrimidination and deamination of cytosine. These all appear to be readily recognized and repaired without mutagenesis. Lindahl considers that other spontaneous lesions involving internal changes in the bases themselves may be important in either giving rise to informational errors or in postmitotic cells allowing an accumulation of damage to be manifested as an increase in single-strand DNA.

Sufficient work has been done, using chemical mutagens, to show that the process of mutagenesis is broadly similar to that with ultraviolet radiation, but details of the repair process differ from one chemical mutagen to another, presumably in relation to the type of

damage to the nucleotide sequence (Lieberman and Forbes, 1973; Kimble *et al.*, 1974; Bouck and di Mayorca, 1976). Direct application of the concept of carcinogenesis as a manifestation of error in DNA replication and repair can be found in work by Loeb *et al.* (1974), who suggest that erroneous base pairing is characteristic of the DNA polymerases of RNA tumor viruses; and by Stich (see Dulbecco, 1977), who found that, using human cells *in vitro*, evidence of DNA fragmentation and DNA repair correlated with carcinogenicity in a large series of chemical agents.

"Spontaneous" mutation rates in bacteria are increased with higher temperatures and can probably be ascribed to thermal agitation. Spontaneous depurination is measurable. Verly *et al.* (1973, 1974) describe a nuclease specific for apurinic sites, and believe that it is part of a repair system specific for such damage. Hastings *et al.* (1976) concluded that much spontaneous mutation in yeasts, as the simplest eukaryotic organisms, arises by mutagenic repair of spontaneous lesions.

The chief impressions in reading accounts of DNA repair and mutagenesis in *E. coli* are the complexity of the process, the existence of alternative pathways for repair, and the large number of distinct enzymes that need to be postulated. Most of the enzyme actions have now been identified as the responsibility of specific proteins, in some cases one protein being involved in two or more activities. According to Drake and Baltz (1976), the polymerase characteristic of phage T4 is both a 5′–3′ polymerase and a 3′–5′ exonuclease equivalent to the "copy editing enzymes" known or postulated for higher systems.

## IV. Xeroderma Pigmentosum

In many ways the most illuminating evidence of the significance of DNA repair in mutagenesis and cancer induction comes from the work on the pathogenesis of xeroderma pigmentosum (XP) initiated by Cleaver in 1969. XP is a rare recessive condition, not geographically or racially restricted, and occurring about once in 250,000 births. The diagnosis is made from the lesions induced in areas of skin and cornea exposed to sunlight. From birth, subjects are highly susceptible to the erythema produced by sunlight or artificial sources of ultraviolet light. Dense freckling develops early, with a wide diversity of pigmented lesions varying in size, shape, and density; moles also appear, and a proportion of malignant melanomas arise at a later stage. The most conspicuous clinical finding is an accelerating appearance of malignant epithelial tumors of the skin, usually seen first between 9 and 12

years of age (Robbins *et al.*, 1974). Some patients have had more than 100 histologically proved cancers removed surgically before the age of thirty. Blindness may result from corneal changes and there is a general atrophy of the skin in exposed areas.

The types of tumor observed are the same as are found on the exposed areas of skin in elderly men who have spent a lifetime in outdoor work in sunny parts of the world. The order of frequency of skin tumors in farmers and other outdoor workers in tropical areas of Australia is basal cell carcinoma, squamous epithelioma, acanthoma, and melanotic sarcoma (Lancaster and Nelson, 1957). This is also the order for the types of tumor that develop on XP subjects, with about 10,000 times the frequency. A proportion of cases—those in complementation groups A and D—show evidence from birth of anomalies of the central nervous system. This is of very great interest, but discussion of the neurological lesions is not relevant in the present context.

Cleaver (1969) reported that cultures of skin cells from XP cases could repair single-strand breaks in DNA produced by X-irradiation in normal fashion but showed gross inefficiency in repairing DNA after ultraviolet exposure. He considered that the deficiency depended on failure of endonuclease to make the first incision in the sequence of enzyme actions needed to remove nucleotides damaged by thymine dimerization.

After a period, when the delay in repair to the DNA of XP cells was ascribed to abnormality in the endonuclease (Setlow *et al.*, 1969), it has been recognized recently by Mortelmans *et al.* (1976) that extracts of standard XP cells have a normal excision capacity for ultraviolet-treated exogenous DNA but are unable to excise pyrimidine dimers from their own chromatin. In other words, the deficiency is in a preexcision step needed to render the damaged DNA accessible to attack.

Further work, largely at the National Institute of Health, Bethesda, but also in many other laboratories, indicated that there was a rather wide range of weaknesses in the excision repair process, any one of which could be associated with the typical syndrome. In the first instance, five complementation groups have been described, four having been recognized in the United States (A, B, C, D) and three (A, C, E) in Europe. Groups A and D contain almost all the patients with concomitant neurological anomalies (Cleaver and Bootsma, 1975).

A number of apparently typical cases gave skin cells that were capable of normal unscheduled DNA synthesis after irradiation with ultraviolet and were referred to as variant XP. Most or all of these cases have allowed demonstration of some weakness in the DNA repair system. Standard XP cell lines show significant deviations from nor-