DAMAGES TO DNA THAT RESULT IN NEOPLASTIC TRANSFORMATION*

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CONTENTS

I. Introduction

A. Why DNA

B. Which changes are biologically important

II. Photoreactivation

A. Application to microbial systems

B. Application to neoplastic transformation

III. Excision repair as an aid in identifying damage

A. The ways to estimate excision

B. Nucleases that recognize damaged DNA

C. Alkylation products and tumors

IV. Conclusions

I. INTRODUCTION

Epidemiological evidence indicates that a large fraction of human cancer arises from environmental chemical agents whose effects are superimposed on genetic predispositions to neoplasia (Cairns, 1975). An important task of basic research is to assess quantitatively the mutagenic and carcinogenic hazards associated with natural and manmade chemicals. The assessment is important because rational decision making requires more than just a determination that an agent is harmful or not. It is important to know how harmful the agent might be. The assessment of hazards is difficult because it is not possible (except in a few - usually accidental - instances) to obtain data for people. We need rules derived from basic research that will permit us to extrapolate from molecules to cells to small animals and thence to man. This brief review emphasizes the first step in the extrapolation process - the molecular nature of damages that result in neoplastic transformation.

A. Why DNA

I shall concentrate on the changes in DNA because there is persuasive experimental evidence for the somatic mutation theory of cancer (Burnet, 1974). The theory derives its strength not only from the clonal analysis of cancer cells but from 2 other important observations. 1) There is a very close correlation between carcinogens and mutagens (Ames, et al., 1973). Eighty percent of chemical carcinogens tested are active in a mutagenic test system (deSerres, 1975) and such test systems are now used for carcinogenesis screening (Loprieno, 1975).

2) Individuals with the disease Xeroderma pigmentosum have very high incidences at early ages of non-melanoma and melanoma skin cancer (Robbins, et al., 1974). The cells from such individuals are defective in one or more systems involved in the repair of ultraviolet (UV) damage to DNA (Setlow and Setlow, 1972; Cleaver, 1974; Sutherland, et al., 1975; Lehmann, et al., 1975). Since many chemical damages mimic UV or ionizing radiation damages (Regan and Setlow, 1974) these observations indicate that unrepaired DNA changes can lead to neoplastic transformation but they do not indicate how they do so. Transformation could arise during either transcription or replication of the damaged template. The latter possibility is analogous to postreplication repair in bacteria since this is supposed to be the error-prone mutagenic pathway (Witkin, 1969).

A comprehensive review of all aspects of repair will be found in Hanawalt and Setlow (1975).

B. Which changes are biologically important

A basic approach to the quantitative estimate of environmental hazards involves knowing the probability per cell that an average nucleotide damage to DNA will result in transformation. The hard part of this analysis is knowing which damages to count as important ones. We do not get this knowledge from dose effect curves alone. As animals or cells or DNA are exposed to increasing doses of chemicals or radiations, there are increasing amounts of all kinds of products, but the most numerous of these may be the least important, as seems to be the case for alkylating agents (see Section III-C).

The conceptual difficulty alluded to above is illustrated in the left side of Fig. 1 which shows that all products increase with increasing dose as does the biological effect - although perhaps with a different dose-response relation than that of products. If we are to identify one of the products as biologically important, we must develop routines to modify experimentally only one of the products to see how changes in this product alone correspond to changes in biological effects. For example, the right side of Fig. 1 shows that during some hypothetical modification process (to be exemplified more completely in Sections II and III) only P_2 decreases concomitantly with the decrease in biological effect. In this case we identify the biological effect with P_2 . Thus an important aspect of the basic investigation of environmental hazards involves the development of experimental procedures to recognize and control the important changes that take place in DNA.

II. PHOTOREACTIVATION

The best understood type of environmental damage, and the one that has led to our understanding of repair in biological systems, is that resulting from UV. The photoproducts formed in DNA are relatively easy to characterize (R. B. Setlow, 1972). Moreover, not only is the major photoproduct cyclobut'diprimidines (pyrimidine dimers) stable to acid hydrolysis and easy to detect in DNA labeled with thymidine, but various photochemical tricks have been used to show that these products are responsible for most of the inactivation of biologically active DNA irradiated in solution (Setlow, 1966, 1968; J. K. Setlow, 1972). Pyrimidine dimers have been shown to be specific substrates for

photoreactivating (PR) enzymes (J. K. Setlow, 1972). The enzyme acts by monomerizing the dimer and converting the DNA back to its original form. The enzyme acts on no other photoproducts. Thus, as illustrated in Fig. 2, the enzyme selectively modifies only one of a number of possible types of damage. Hence, if we observe a biological phenomenon that shows all the attributes of enzymic PR, we describe it as resulting from the formation of pyrimidine dimers in DNA as a result of UV.

A. Application to microbial systems

PR activity is found among almost all phyla investigated (J. K. Setlow, 1972; Rupert, 1975). In many systems, the activity is able to reverse a substantial fraction of the deleterious effects of UV irradiation. For example, in *E. coli* PR reverses 80% of the killing effect and between 70 and 90% of the changes in DNA that lead to reversion to prototrophy corresponding to several different suppressor pathways (Person, et al., 1975). Mutation of T4 phage by irradiation with near UV in the presence of a sensitizer (a process that greatly enhances the yield of thymine-thymine dimers compared to other products) is almost completely photoreversible (Meistrich and Drake, 1972). The fact that many different types of mutational changes such as AT to GC, GC to AT, and frameshifts are photoreversible indicates that the process that results in the fixation of mutation in daughter DNA does not necessarily involve a change exactly opposite the thymine-thymine dimer in parental DNA.

B. Application to neoplastic transformation

Ultraviolet radiation is carcinogenic (Blum, 1959), but because UV makes many photoproducts (see Fig. 2) it is not apparent what role of pyrimidine dimers play in the process. Hart and I (1975) have carried out an experiment using fish and PR in an attempt to resolve this uncertainty. We used small fish, Poecilia formosa, that may be grown in clones. Thus cells from one fish may be injected into another without danger of rejection. The fish contain high levels of PR enzyme and hence the experimental design shown in Fig. 3 was followed. Cells - mostly thyroid - from a number of fish were irradiated in vitro with 254 nm radiation. The cells were injected back into isogenic recipients and many the recipients, when observed six to nine months later, had thyroid carcinomas. At an incident fluence of 200 ergs/mm^2 , and 2×10^5 cells injected per fish, all the recipients had carcinomas. If the UV irradiated cells were first exposed to photoreactivating illumination before injection, less than 10% of the recipients had tumors. As illustrated in Fig. 3, the order in which the UV and PR were delivered is important. If PR is given before the UV, it has no effect on the subsequent development of tumors. These data are strong evidence for the involvement of pyrimidine dimers in tumor development in fish. We estimate that the probability per dimer of transforming a cell is 10^{-10} .

III. EXCISION REPAIR AS AN AID IN IDENTIFYING DAMAGE

Cells able to repair damage to their DNA are able to survive better and have fewer mutations (and presumably neoplastic transformations) per unit

dose because there is less damage to be fixed, or virus induced, in subsequent replications or transcriptions.

Damages that distort the DNA double helix (Cerutti, 1975) would be expected to interfere with the fidelity of replication and translation. Since in many biological systems such alterations are subject to excision repair, any indication of such repair itself is taken as evidence for damage to DNA, even though the detailed nature of the damage may not be known. If some types of cells do excision repair and others do not, then excision is an appropriate modification of damage illustrated in Fig. 1, and it is appropriate to compare the biological effects of such damage in the two cell classes. Damage that is repaired slowly would be anticipated to have larger biological effects than damage that is repaired rapidly. Of course, damages that are not subject to excision repair in any cell systems investigated must be analyzed by other means for their deleterious or innocuous character.

A. The ways to estimate excision

A number of experimental ways of detecting excision repair are shown in Table I. The details of these techniques are discussed elsewhere (Regan and Setlow, 1973; Cleaver, 1974; Setlow, 1975). Only the first technique is a direct measure and is able to identify the actual damage excised. The others are more general, do not require knowledge of the molecular nature of the damage, and are usually easier to do than the first. However, by themselves they cannot identify the damage. The loss of endonuclease sensitive sites is a good example. This method uses exogenous damage-specific endonuclease (Grossman, et al., 1975) to treat the DNA of cells that have been damaged by chemical or physical agents.

If damages exist, the endonuclease makes a large number of single strand breaks in the DNA and they can be detected by a number of techniques - a common one being the decrease in sedimentation rate in alkali. If the damage is excised before the DNA is treated with the endonuclease, very few breaks will be observed since the endonuclease step is the first enzymatic step in excision.

A number of attempts have been made to isolate repair endonucleases from cells. The dimer-specific nucleases (Table II) make one single strand break per dimer. A number of endonucleases have been isolated that act on UV irradiated DNA but the number of single strand breaks made is often minute compared to the number of dimers. These endonucleases do not work on dimers. The nature of the photoproducts worked on is not known, but obviously these products are a minor component of the total number. Thus, the role of these endonucleases in repair of UV damage may be unimportant.

B. Alkylation products and tumors

The major alkylation products in DNA are 7 alkylguanines, but these not only seem to have little biological significance but are excised at negligible rates (Lawley, 1975; Singer, 1975; Strauss, et al., 1975). The mutagenic product seems to be a minor one - O⁶ alkylguanine. Two types of experiments suggest that this product may also be an important carcinogenic lesion. The first (Maitra and Frei, 1975) measures the type and amounts of alkylation products in the tissues of mice treated with methylating agents that are tumorigenic (methylnitrosourea, MNU) or non-tumorigenic (methylmethanesulfonate, MMS). The data in Table III indicate that there is 10-fold more O⁶ MeG in the DNA of mice treated with MNU than in mice treated with MMS.

The second line of evidence (Goth and Rajewsky, 1974) concerns the distribution and half-life of alkylation products in the tissues of rats treated with ethylnitrosourea (ENU). Treatment with the agent results in brain tumors and the data in Table IV indicate that although there is little difference in the amounts of product observed in brain and in liver, O^6 EtG persists in brain for a much longer time than in liver. The other alkylation products detected had approximately equal half lives in the two tissues. Table IV also points out that other products - triesters - were not determined and that they may account for 70% of the alkylation products. Their half lives obviously must be known if a reasonable conclusion is to be drawn from such experiments.

IV. CONCLUSIONS

The determination of the probability that particular changes in DNA will give rise to tumors is a difficult one and has been estimated only for UV irradiation. Nevertheless, such determinations are important if we are to evaluate the long range hazards of environmental agents. There are no strict rules as to how to solve this general problem, but a lot of ingenuity will have to go into the solutions.

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Table I

Direct and Indirect Measures of Excision Repair of DNA^a

1. Specific loss of products
2. Loss of endonuclease-sensitive sites
3. Unscheduled DNA synthesis
4. Repair replication
5. Photolysis of bromouracil incorporated during repair
6. Host cell reactivation of damaged virus DNA

^aDetail concerning these techniques can be found in Cleaver (1974), Regan and Setlow (1973), and Setlow (1975).

Table II

Nucleases that Act on UV-irradiated DNA

	Source	Analytical ^a Methods	References
Specific for dimers			
	<u>M. luteus</u>	1,2	Carrier and Setlow, 1970 Grossman, et al., 1975
	<u>E. coli</u>	1,3	Grossman, et al., 1975
	T4 phage	1,2	Friedberg, 1975
Unknown specificity ^b (do not act on dimers)			
	HeLa, XP	1,2	Bacchetti, 1975
	HeLa	2	Brent, 1972
	HeLa, WI-38, XP	2	Duker and Teebor, 1975
	rat liver	2	VanLanker and Tomura, 1974
	<u>Dictyoselium discoideum</u>	2	Deering and Jensen, 1975
	<u>B. subtilis</u>	2	Hayase, 1975
	<u>H. influenzae</u>	2	Small, 1975

^a1) enzymic photoreactivation; 2) number of endonuclease induced nicks; 3) binding assay.

^bthe numbers of endonuclease-sensitive sites is much less than the numbers of dimers.

Table III

Percentage Distribution of Products in
DNA of Treated Mice^a

	MMS		MNU	
	Bone marrow	lung	Bone marrow	lung
<u>Product</u>				
7 MeG	89	92	87	90
3 MeA	11	8	6	6
O ⁶ MeG	0.4	0.5	6	4

^aPercentages calculated from data of Maitra and Frei (1975). Swiss mice were injected with MMS (non-tumorigenic) and MNU (tumorigenic) to give approximately equal levels of methylation. The distributions shown are for 2h after injection. Similar values were observed in other tissues. No loss of O⁶ MeG was observed relative to 7 MeG in 12h.

Table IV

Amounts and Persistence of Products in DNA
of Rats Treated with ENU^a

	Relative amount ($\times 10^6$)		approximate half life (h)	
	brain	liver	brain	liver
0 ⁶ EtG/G	10	16	220	30
3 EtA/A	5	9	16	12
7 EtG/G	24	32	90	75
triesters ^b	N o t d e t e r m i n e d			

^aData from Goth and Rajewsky (1974). Treatment with ENU results in brain tumors but not in liver tumors.

^bSun and Singer (1975) showed that 70% of the alkylation products in the DNA of ENU-treated HeLa cells were triesters.

FIGURE LEGENDS

- Fig. 1. A schematic to illustrate the appearance of DNA products and a biological effect as a function of radiation or chemical dose, and that a routine to modify the biological effect might modify only one product and so relate that product alone to the biology.
- Fig. 2. A diagram showing some of the known photoproducts arising from UV-irradiation of DNA (T^* : altered thymine; $\hat{T}T$: thymine-thymine dimer; $\hat{C}T$: cytosine-thymine dimer; $\hat{C}C$: cytosine-cytosine dimer; U: uracil derived from the deamination of cytosine; A^* : altered purine; ----protein: DNA protein crosslink). Photoreactivation monomerizes the dimers and does not affect the other products.
- Fig. 3. A diagram of an experiment (Hart and Setlow, 1975) to determine the role of pyrimidine dimers in the induction of neoplastic transformation in fish cells. The large difference between the effects of UV + PR and PR + UV indicates that most of the tumors arise from dimers. Each line in the figure represents approximately 100 fish and "many tumors" means most of the fish had a thyroid carcinoma.

Fig. 1

PHOTOREACTIVATION MONOMERIZES PYRIMIDINE DIMERS

Fig. 2

Fig. 3