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CARCINOGENESIS, DNA REPAIR AND CHROMATIN

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Summary

1) A DNA damage is not a mutation; the damage must be processed by several cycles of DNA replication before it eventually becomes a mutation;
2) Most DNA damages, which are potentially mutagenic and carcinogenic, are repaired;
3) A competition between repair and DNA replication fixes the mutation rate. An established mutation can no longer be repaired;
4) DNA repair is the first line of defense against cancers due to physical and chemical mutagens. Repair deficiencies are associated with a high incidence of cancers;
5) In eucaryotic cells, repair enzymes have been located in chromatin non-histone proteins;
6) Native chromatin exhibits little activity on an added damaged DNA. The non-histone proteins must be separated to demonstrate the full activity of the enzymes on such a substrate;
7) DNA repair *in vivo* might depend not only on repair enzymes but also on other chromatin proteins necessary for the correct orientation of the enzymes to work on chromatin DNA;
8) We have observed a release of O^6-ethylguanine, a modified base thought to be mutagenic and carcinogenic, from isolated nuclei treated with ethylnitrosourea. This result suggests that the first enzyme involved in the repair of this carcinogenic damage might belong to the class of DNA glycosylases.

Epidemiological data point to environmental factors as major causes of human cancers. It is usually accepted that chemical and physical agents produce carcinogenic mutations in cell DNA; some chemical agents which are indirect carcinogens must be metabolically

activated before they can react with DNA.

The carcinogenic mutation would be an ordinary mutation that appears in a target, the alteration of which leads to malignant transformation. We shall thus examine the two general mechanisms leading to mutations.

1) A minor base alteration which changes the pairing properties of the base without blocking the DNA polymerase

An example is methylation or ethylation of guanine O^6 which has the consequence that the complementary base is no longer cytosine but thymine [1,2]. The O^6-alkylguanine is only a *promutation;* two DNA replications are necessary to make a mutation from the promutation (figure 1a). The minor modification of the purine does not block the DNA polymerase of the replication fork and the enzyme puts thymine in the new strand in front of the O^6-alkylguanine of the template strand. The next replication cycle places adenine in front of thymine. So one of the four molecules of the second generation contains an AT instead of a GC pair; this change in the genetic information is the mutation. The mutation is hereditary from then on since the mutated DNA contains only normal and correctly paired bases; in sharp contrast with the promutation, the mutation can no longer be repaired (see later).

2) A major DNA lesion which blocks the DNA polymerase

Examples are pyrimidine dimers produced by UV irradiation, bulky groups attached to bases, etc... Such a lesion blocks the DNA polymerase of the replication fork which stops forming the undertaken Okazaki piece and resumes its activity only at the beginning of the next one; this leaves in the new strand a gap [3,4] which is lethal for the cell unless it is filled. the filling of the gap necessitates the synthesis of new proteins and is made in one of two ways: by recombination using a piece of the same polarity strand from the sister molecule or by SOS repair. This SOS repair enables the DNA polymerase to terminate the interrupted Okazaki piece, passing the bulky lesion and placing most often wrong bases in front of it (figure 1b); this mistake in the new strand is a source of mutation [5,6].

We see that, in the two instances (promutation or lethal damage inducing SOS repair), a replication of DNA is necessary for the appearance of the mutation. to prevent the mutation, DNA must be repaired before it replicates; this repair is an excision of the promutation or of the lethal damage. Two kinds of pre-replicative excision repair have been distinguished:

1) Nucleotides excision: the lesion is recognized by a repair endodeoxyribonuclease. It is the case with pyrimidin dimers produced by UV irradiation (figure 2a). A UV0endonuclease cuts the strand carrying the dimer on the 5' side of this lesion; a 5'→3'

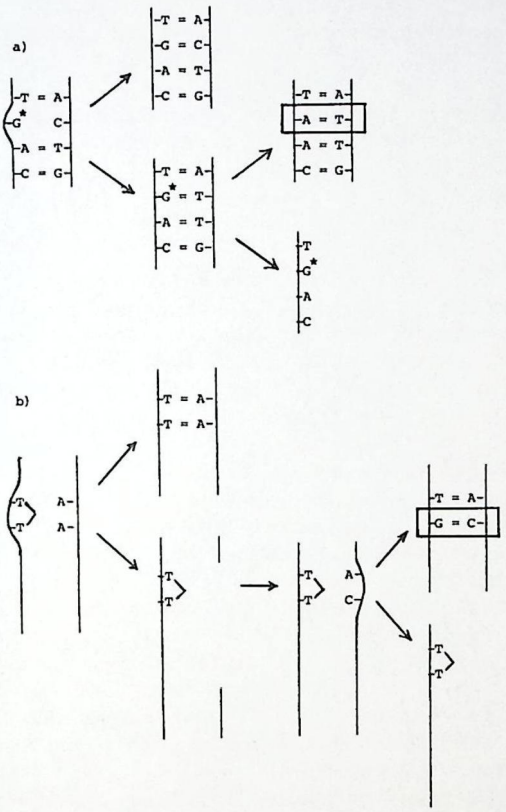

Figure 1: The two mechanisms of mutagenesis

a) The mutagen has alklated a guamine on O^6 (G*); the complementary base in no longer C (deformation of the molecule) but T. When DNA replicates and that the strand containing G* is used as template, DNA polymerase places T in front of the modified purine. At the next replication cycle, the enzyme places and A in front of this T. The pair AT (within the frame) is the mutation; the original DNA molecule contained GC at this place.

b) The pyrimidine dimer (T⌒T), produced by UV irradiation, distorts the double helix. When DNA replicates, the lesion blocks the DNA polymerase and a lethal gap appears in the new strand. But induction of SOS repair allows the DNA polymerase to fill the gap placing any bases in front of T⌒T (A and C, for instance). At the next replication cycle, DNA polymerase puts complementary bases in front of these randomly chosen bases completing the formation of the mutation (with the frame).

exonuclease excises the dimer in an oligonucleotide; a DNA polymerase synthesizes the missing segment using the other intact strand as template; a ligase binds the new piece to the rest of the molecule [7].

2) Base excision: the modified base is removed by a DNA glycosylase [8] leaving an AP (apurinic or apyrimidinic) site which is recognized by an AP endodeoxyribonuclease [9]; this enzyme cuts the DNA strand on the 5' side of the AP site [10]. The repair then proceeds as above.

In normal cells, most of the DNA lesions, promutations or lethal damages, are repaired. Pre-replicative repair erases the damage so that no mutation can appear; but, on the other hand, if DNA replication occurs before the lesion is excised, a mutation is formed which is no longer reapairable. The mutation rate thus depends on a competition between pre-replicative excision repair and DNA replication.

The repair pathways have been studied mostly in bacteria, but we have many reasons to believe that pre-replicative excision repair and SOS repair are also working in eucaryotic cells and, in particular, in mammalian cells where they are relevant for the problem of carcinogenesis:

- carcinogenic lesions, like O^6 alkylguanine, are excised from DNA;
- competition between pre-replicative excision repair and DNA replication plays a role in the formation of cancers: a single dose of dimethylnitrosamine to a rat does not produce a liver cancer unless the animal is also subtotally hepatectomized [11]; in this case, the stimulated DNA replication occurs before the carcinogenic damages might have been repaired;
- DNA repair influences the tissue localization of cancers: the brain localization of tumors produced by ethyl-or methylnitrosourea in young rats has been correlated with an inefficient O^6-alkylguanine excision from brain DNA [12,13];
-diseases where the excision repair system is deficient (like xeroderma pigmentosum, ataxia-telangiectasia, Fanconi's anemia, etc...) are associated with a very high incidence of cancers [14,15]. It might even be possible that heterozygotes, which do not show the usual symptoms of the disease, would nevertheless be more sensitive to carcinogens than individuals not carrying a mutated gene.

It would seem that the frequency and tissue localization of cancers depend on:

1) nature of the carcinogen and amount introduced in the organism;
2) localization, activity and inducibility of activation enzymes if it is an indirect carcinogen;
3) rate of pre-replicative excision repair;
4) mitotic index in the tissue (probability of entering the S phase for cells containing unrepaired carcinogenic damages);
5) inducibility of the SOS repair if the DNA contains a bulky lesion.

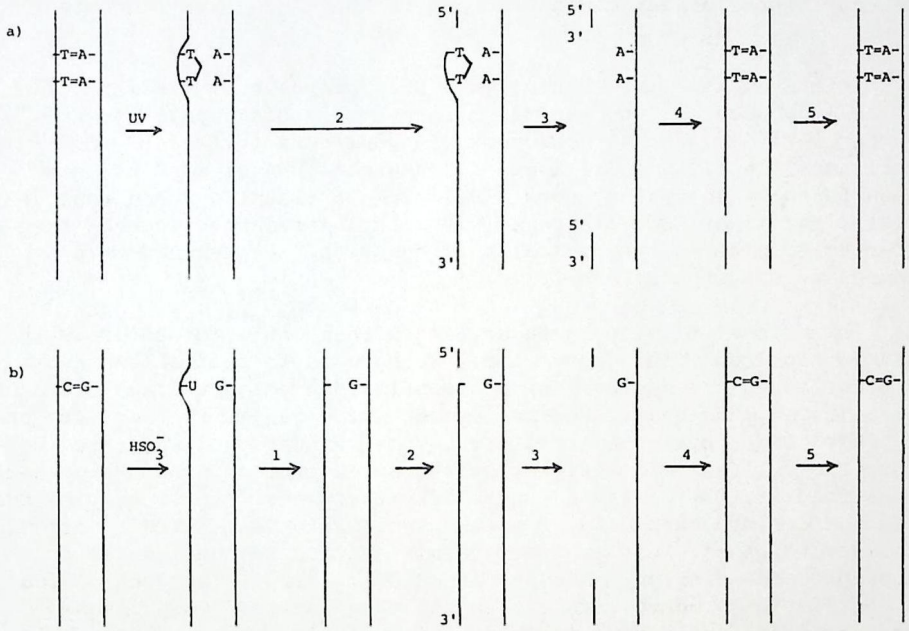

Figure 2: Pre-replicative excision repair

a) Nucleotide excision: UV irradiation produces a pyrimidine dimer (T⌒T). The UV endonuclease nicks the strand on the 5' side of the dimer (2). An exonuclease excises the dimer in an oligonucleotide and removes a few other nucleotides (3). Starting from the 3' end of the gap, DNA polymerase makes a new piece using the intact opposite strand as template (4). Ligase binds the piece at the 5' end of the gap.

b) base excision: Bisulfite deaminates cytosine into uracil. Uracil DNA glycosylase removes the uracil leaving an AP site (1). An AP endonuclease nicks the strand on the 5' side of the AP site (2). The rest as above.

Even if it saves the cell, the SOS repair is highly noxious as far as carcinogenesis is concerned: it is much better for the organism that the damaged cell dies rather than having a probability to become malignant.

The greater part of carcinogenic DNA lesions are repaired so that pre-replicative excision repair is not only the first line of defense of the organism against cancers but also a very important one.

If the excision of carcinogenic DNA damages in mammalian cells is well documented, the enzymic mechanism of this repair has still to be clarified. Repair endodeoxyribonucleases (UV and AP endonucleases [16,17,18]) are known and a uracil DNA glycosylase has been found by several athuors [19,20,]. We recently found that isolated rat liver nuclei treated with ethylnitrosourea release free 0^6-ethylguanine in the medium; this suggests the presence of a competent DNA glycosylase [21].

The main difficulty, when dealing with eucaryotic cells, is that DNA is repaired within chromatin. We have shown that AP endodeoxyribonucleases are nuclear enzymes mostly located in the non-histone proteins of chromatin. But native chromatin has little activity on an added DNA containing AP sites; chromatin must be dissociated before the full enzymic activity can be demonstrated on this exogenous substrate [22,23,]. It is very likely that other repair enzymes are also built into chromatin to work on chromatin DNA. With eucaryotic cells, one cannot just look for repair enzymes in homogenates or supernatants using an added modified DNA; there is a risk to find a degradation product rather than the true enzyme. Comparisons made between normal and repair-deficient cells using these techniques have probably little biological significance and their conclusions might even be misleading. What is important are the chromatin enzymes and how efficiently they work on chromatin DNA. It is moreover quite possible that other chromatin proteins besides the enzymes are important for DNA repair; these proteins might be needed to bind the enzyme in chromatin or to give it a correct orientation to work on chromatin DNA. Chromatin is cell extract from xeroderma pigmentosum-suffering patients, group A, does not excise pyrimidine dimers from its DNA although the extract contains a UV-endonuclease; excision occurs however when an extract of normal cells is added which suggests that normal cells have an XP factor (inactive in diseased cells) which is necessary for the UV-endonuclease activity on chromatin DNA [16].

References

1) Loveless, A.; Nature, 1969, 223, 206-207.

2) Gerchman, L.L. and Ludlum, D.B.; Biochem. Biophys. Acta, 1978, 308, 310-316.

3) Smith, K.C. and Meun, D.H.C.; J. Mol. Biol., 1970, 51, 459.

4) Johnson, R.C.; Nature, 1977, 267, 80-81.

5) Witkin, E.M.; Proc. XII Intern. Cong Genetics, 1969, 3, 225-245.

6) Radman, M., in Molecular Mechanisms for Repair of DNA (Hanawalt, P.C. and Setlow, R.B., ed.), Plenum Publ. Corp., New York, part A, p. 355-367.

7) Hanawalt, P.C.; Endeavour, 1972, 31, 83-87.

8) Lindahl, T.; Nature, 1976, 259, 64-66.

9) Verly, W.G. and Paquette, Y.; Can. J. Biochem., 1972, 50, 217-224.

10) Gossard, F. and Verly, W.G.; Eur. J. Biochem., 1978, 82, 321-332.

11) Craddock, V.M.; J. Natl Cancer Inst., 1871, 110, 39-47.

12) Goth, R. and Rajewsky, M.F.; Proc. Natl Acad. Sci. (US), 1974, 71, 639-643.

13) Margison, G.P. and Kleihues, p.; Biochem. J., 1975, 148, 521-525.

14) Bootsma, D.; VII Intern. Congress Photobiol., Rome 1976, S 45.

15) Setlow, R.B.; Nature, 1978, 271, 713-717.

16) Mortelmans, K., Friedberg, E.C. Slor, H., Thomas, G. and Cleaver, J.E; Proc. Natl Acad. Sci (US), 1976, 73, 2757-2761.

17) Verly, W.G. and Paquette, Y.; Can. J. Biochem., 1973, 51, 1003-1009.

18) Ljungquist, S. and Lindahl, T.; J. Biol. Chem., 1974, 249, 1530-1535.

19) Sekiguchi, M., Hayakawa, H., Makino, F., Tanaka, K. and Okada, Y.; Biochem. Biophys. Res. Comm., 1976, 73, 293-299.

20) Kuhnlein, U., Lee, B. and Linn, St.; Nucleic Acids Res., 1978, 5, 117-125.

21) Renard, A., Thibodeau, L. and Verly, W.G.; Fed. Proc., 1978.

22) Thibodeau, L. and Verly, W.G.; J. Biol. Chem., 1977, 252, 3304-3309.

23) Thibodeau, L. and Verly, W.G.; J. Supramolecular Structure, 1978, suppl. 2, p. 57 (abs 126).