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THE O^6-ALKYLGUANINE TRANSFERASE ACTIVITY OF RAT LIVER CHROMATIN

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O^6-Methylguanine (O^6-meG) and O^6-ethylguanine (O^6-etG) disappear from liver DNA of rats treated with nitrosamines or nitrosoureas. The proteins of rat liver have an activity that eliminates O^6-meG and O^6-etG from an alkylated DNA used as a probe [1,2]. It is uncertain if the activity found in whole cell extracts is the one and only the one acting on nuclear DNA in the living cells. Careful work on Mer$^-$ cells might help to bridge the gap between *in vivo* and *in vitro* studies [3,4].

When we started to work on the repair of O^6-alkylguanine lesions in nuclear DNA several years ago, we used another approach : we studied the repair in isolated nuclei alkylated *in vitro* [5]. Repair of cell DNA in isolated nuclei is much closer to DNA repair that occurs *in vivo* than repair of a probe DNA with isolated proteins.

ISOLATED NUCLEI ETHYLATED *in vitro* [5,6]

Repair of O^6-etG and 7-etG (7-ethylguanine) Lesions in Nuclear DNA.

Nuclei were isolated from rat liver cells and treated *in vitro* with labelled ethylnitrosourea (ENU) during 30 min at 37° before measuring the 7-etG and O^6-etG contents of the nuclear DNA. In spite of a repair of 7-etG DNA lesions in the incubated nuclei (see later), the O^6-etG/7-etG ratio in nuclear DNA was lower than in a control of naked DNA similarly treated. The results indicated that O^6-etG DNA lesions were repaired in isolated nuclei; we calculated that the amount of repair had the same magnitude as the *in vivo* repair.

In isolated nuclei, the nuclear DNA remains in its natural environment; DNA repair in isolated nuclei must be performed by the same repair system as the one acting *in vivo*. Since the repair has the same magnitude *in vivo* and in isolated nuclei, we conclude that all the necessary factors to repair O^6-etG lesions in nuclear DNA are located in the cell nucleus.

There was no ENU left at the end of the first 30-min incubation at 37°; when the incubation was prolonged for another 30 min, there was little additional decrease of O^6-etG content of the nuclear DNA; this is in agreement with the present knowledge of a limited amount of ethyl acceptor playing a role in the repair.

On the other hand, between 30 and 60 min of incubation, the 7-etG content of nuclear DNA decreased. The 12-h half-life in DNA calculated for this ethylated base is too short to be only the result of a spontaneous depurination; there must be, in addition, a repair mechanism for 7-etG lesions in nuclear DNA.

Repair Mechanism of O^6-etG Lesions in Nuclear DNA.

In vivo studies do not give any indication on the repair mechanism. Our experiments with isolated nuclei ethylated *in vitro* led us to eliminate some possibilities.

We looked for O^6-etG in the incubation medium. We did not find any, either as a free base or in oligonucleotides.

On the other hand, free 7-etG was found. The amount after 60-min incubation was about twice the amount after 30 min, and the amount which appeared between 30 and 60 min was equal to the 7-etG lost by the nuclear DNA during this period. We previously concluded that a repair of 7-etG DNA lesions ought to exist in addition to the spontaneous depurination; since 7-etG is released in the medium as a free base, it seems that the repair involves a DNA glycosylase.

The incubation medium of the ethylated nuclei contained free 7-etG but no free O^6-etG; we conclude that the repair of O^6-etG lesions in nuclear DNA is not due to a DNA glycosylase.

We did not find any radioactive oligonucleotides, enriched or not in O^6-etG, in the incubation medium. No conclusion can be drawn however from this observation since the nuclear membranes might be impermeable to oligonucleotides.

THE REPAIR ACTIVITY OF THE CHROMATIN PROTEINS

Cellular Distribution of the Repair Activity [7].

The repair activity was measured with a probe DNA alkylated with ENU. About 80 % of the repair activity was found in the nucleus and most of it (65 % of the total cellular activity) was in chromatin. This repair activity is toward a probe DNA and some of it (for instance the activity localized in the cytoplasm) might not participate to the repair of nuclear DNA *in vivo*. An interesting question that we have not yet solved is whether or not the repair factors in other cell compartments are identical with the chromatin repair factor which is likely the one involved in the repair of nuclear DNA.

The rest of this paper will describe the work done with the chromatin proteins.

Mechanism of the Repair of O^6-etG Lesions in a Probe DNA with Chromatin Proteins.

The chromatin proteins are prepared in the following way : cell nuclei are isolated from rat liver; the purified nuclei are swollen in a hypotonic medium and the nuclear membranes are torn apart; the chromatin, collected by centrifugation, is dissociated with heparin-Sepharose and the complex is extracted with 0.3 M KCl. This extract does not contain histones or DNA [7].

When these chromatin proteins are incubated with DNA alkylated with ENU, the O^6-etG disappears from DNA. The extent of repair is of the same magnitude as the repair observed *in vivo* or in the nuclei ethylated *in vitro*, suggesting that the repair activity of the chromatin proteins might be responsible for the repair of nuclear DNA *in vivo*.

No free O^6-etG can be found in the incubation medium : the repair activity of chromatin proteins acting on a probe DNA as the repair activity in isolated nuclei acting on nuclear DNA, is not due to a DNA glycosylase. The oligonucleotides which appear in the medium are not enriched in O^6-etG so that the repair activity of the chromatin proteins is not the result of the consecutive action of a specific endonuclease and an exonuclease [7].

When the DNA alkylated with labelled ENU is hydrolyzed to nucleosides, HPLC analysis separates numerous radioactive peaks; one of them is O^6-ethyldeoxyguanosine.

This peak disappears during incubation with the chromatin proteins. There is little modification of the other peaks and no new peak appears : the O^6-etG is thus not changed into another ethylated residue that would remain attached to the sugar-phosphate backbone of the DNA [8].

The ethylated DNA and the chromatin proteins were separated by isopycnic centrifugation in CsCl. At O time, all the radioactivity is in the DNA band; after 2-h incubation at 37°, part of the radioactivity is found in the protein band [8]. About 20-30 % of the radioactivity in the protein band is due to oligonucleotides [9], but most of it is in proteins as can be shown by digestion with trypsin or proteinase K.

Tryptic digestion of the chromatin proteins incubated with ethylated DNA followed by HPLC analysis gives two radioactive oligopeptides [8] which are ultimate products of digestion; further trypsin treatment does not turn one into the other [9]. When these oligopeptides are further hydrolyzed with pronase and leucine aminopeptidase, the radioactivity is found in S-ethylcysteine [8].

Digestion of the reacted chromatin proteins with proteinase K also gives two radioactive oligopeptides that change with time; part of the radioactivity finally appears in S-ethylcysteine [9].

The repair activity of the chromatin proteins from rat liver is thus a transalkylase : the ethyl group is moved from DNA O^6-etG onto cysteine residues of acceptor proteins. The existence of two different radioactive oligopeptides, which is particularly evident after trypsin digestion, suggests that there are two different acceptors or that one acceptor can be alkylated on two different cysteines.

Transalkylation appears to be the only important mechanism of O^6-etG repair in the *in vitro* system with chromatin proteins. The repair can be followed in two ways : disappearance of O^6-etG from DNA; ethylation of the acceptor protein. An experiment was carried out in which the two assays were run in parallel : one consisted in acid depurination of DNA followed by HPLC analysis; for the other, the proteins were digested with proteinase K and the DNA precipitated with CTAB (cetyltrimethylammonium bromide) before measuring the radioactivity of the supernatant. The two methods gave essentially the same results which suggests that transethylation on chromatin proteins is the only significant pathway of O^6-etG repair [9].

Purification of the Repair Activity of the Chromatin Proteins and of the Ethylated Acceptor.

The specific repair activity toward O^6-etG lesions in a probe DNA is 50 times higher in chromatin proteins than in whole cell proteins [7]. A 30-fold additional purification is achieved by successive chromatographies on DEAE-Trisacryl, Biogel P-60 and carboxymethyl-Trisacryl (Henrard, unpublished). The repair factor has a molecular weight of about 25,000.

The alkylated acceptor is strongly retained on DNA : a concentration of 3.5 M guanidinium chloride is necessary to separate them. Fractogel analysis gives a molecular weight of about 25,000 (Lemaître, unpublished).

Repair of O^6-Methylguanine (O^6-meG) Lesions in a Probe DNA.

The repair of O^6-meG DNA lesions with chromatin proteins follows the same mechanism as the repair of O^6-etG DNA lesions. The same alkyl acceptor is used in both repairs as shown in the following experiments [10].

The chromatin proteins are incubated with an excess of methylated DNA until no more O^6-meG disappears; addition of ethylated DNA is not followed by any removal of O^6-etG. The experiment where ethylated DNA is added first and methylated DNA only afterwards leads to symmetrical results. If no alkylated DNA is present in the medium during the first incubation, the repair during the second incubation occurs to a normal extent showing that the previous results are not due to an instability of the repair systems.

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