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Inducible responses to DNA damages

Repair of AP sites in DNA.

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Résumé.

*L'endonucléase VI d'*Escherichia coli *est une désoxyribonucléase spécifique pour les sites AP (apuriniques ou apyrimidiques). Elle coupe la liaison phosphodiester immédiatement voisine du site AP, du côté 5' de celui-ci, donnant naissance à des extrémités 3'-hydroxyle et 5'-phosphate. Le DNA contenant des sites AP peut être complètement réparé* in vitro *à l'aide de l'endonucléase VI, la DNA polymérase I et la ligase ; les détails du mécanisme de réparation sont connus.* E. coli *contient d'autres AP endonucléases, certaines non spécifiques, certaines coupant du côté 3' du site AP.*

La plus grande partie de l'activité AP endonucléasique du foie de rat se trouve dans la chromatine. On trouve cependant des AP endonucléases dans d'autres compartiments cellulaires ; ces autres AP endonucléases pourraient être des précurseurs de l'enzyme chromatinien.

L'AP endonucléase chromatinienne coupe du côté 5' du site AP. Du DNA contenant des sites AP peut être réparé in vitro *en utilisant uniquement des enzymes extraits de la chromatine : AP endonucléase, exonucléase 5'-3', DNA polymérase β et ligase.*

Summary.

Escherichia coli *endonuclease VI is a deoxyribonuclease specific for AP (apurinic or apyrimidinic) sites ; it cleaves the phosphodiester bond immediately neighbouring the AP site on its 5' side leaving 3'-hydroxyl and 5'-phosphate ends. DNA with AP sites can be repaired* in vitro *with endonuclease VI, DNA polymerase I and ligase ; the repair mechanism is described.* E. coli *has other AP endonucleases ; some of them are not specific for AP sites and some of them cut 3' to the AP sites.*

Most of the rat liver AP endonuclease activity is in chromatin. Some is however found in other cell compartments and it has been speculated that these enzymes might be precursors of the chromatin enzyme.

The chromatin AP endonuclease is specific for AP sites ; it cuts 5' to the AP site. DNA with AP sites can be repaired in vitro *with enzymes purified from chromatin : AP endonuclease, 5'-3 exonuclease, DNA polymerase β and ligase.*

An endonuclease specific for AP (apurinic or apyrimidinic) sites in DNA was first described in *E. coli* [1]. It was soon evident that enzymes with the same specificity were present in all cells - bacterial, animal or plant cells [2].

The *E. coli* enzyme, later named endonuclease VI, was purified to homogeneity [3]. It was shown to hydrolyze a phosphodiester bridge 5' to the AP site leaving 3'-OH and 5'-phosphate ends [4]. Using the *H. infuenzae* homologous enzyme, it was demonstrated that the phosphodiester bridge hydrolyzed is the immediate neighbour of the AP site [5]. Exonuclease III, which is a 3'-phosphatase and a 3'-5' exonuclease working from nicks on double-stranded DNA, is the same protein as endonuclease VI [6]. T_7 DNA containing AP sites was completely repaired *in vitro* with endonuclease VI, DNA polymerase I and ligase [7]. The details of the repair have been worked out : endonuclease VI incises the damaged strand 5' to the AP site and its exonuclease III activity widens the nick into a gap, but leaves the AP site in DNA ; DNA polymerase I fills the gap, excises the AP site in a di- or tri-nucleotide by its 5'-3' exonuclease activity, then catalyzes the translation of a nick which is eventually sealed by ligase [8, 9].

E. coli has a second endonuclease specific for AP sites called endonuclease IV [10] ; it also cuts

5' to the AP site [11]. Endonuclease III [12], which has a broader spectrum of actions, cuts 3' to the AP site leaving 3'-OH and 5'-phosphate ends [11]. A double cut, 3' and 5', which releases the AP site as deoxyribose phosphate, reduces the probability that DNA polymerase I would produce strand displacement rather than nick translation [13].

AP endonucleases have been found in other bacteria and in plants, and purified ; no attempt will be made to survey these topics. AP endonucleases have been found in many kinds of animal cells ; we shall briefly discuss the results with rat liver.

AP endonuclease activities have been found in chromatin, nuclear sap, cytoplasm, nuclear and cytoplasmic membranes, and mitochondria of rat liver [14]. More than 90 per cent of the total activity is in chromatin ; it is however not directly demonstrable with foreign depurinated DNA : the chromatin must be dissociated and the AP endonuclease activity is found in the non-histone proteins. This result suggests a highly organized structure orienting the chromatin repair enzyme to work on nuclear DNA [14].

The chromatin AP endonuclease has been purified. Two species are separated on hydroxyapatite ; one is eluted with 0.2 M, the other with 0.3 M phosphate. The « 0.2 M » isozyme has been completely purified [15] ; it hydrolyzes the phosphodiester bridge which is the immediate neighbour of the AP site on its 5' side leaving 3'-OH and 5'-phosphate ends [16]. The ratio « 0.2 M »/« 0.3 M » isozymes varies with the preparation of the AP endonuclease ; when protease inhibitors are used and the time until the end of the hydroxyapatite chromatography is kept to a minimum, the ratio can be as low as 8 per cent. It seems that the « 0.3 M » species is the true chromatin enzyme and that most of the « 0.2 M » species is an artifact. The « 0.3 M » AP endonuclease was also purified ; it has the same molecular weight of about 42,000 as the « 0.2 M » isozyme. The two isozymes cut the same phosphoester bond 5' to the AP site (unpublished).

Nuclear sap and cytosol enzymes are likely the same protein ; the activity is much more concentrated in the nuclear sap than in the cytoplasm [14]. The nuclear sap enzyme appears different from the main chromatin AP endonuclease. Nuclear membrane and cytoplasmic membrane enzymes are probably the same, but they are different from the nuclear sap and chromatin enzymes ; the membrane enzymes are highly stimulated with 0.5 per cent Triton X-100 [14]. The membrane enzyme behaves as an intrinsic membrane protein (unpublished).

It has been speculated that the different cellular AP endonucleases might be the product of a single gene at different stages of maturation [14]. The precursor of the chromatin enzyme synthesized on free ribosomes might have a C-terminal hydrophobic end so that it attaches to the cytoplasmic side of the endoplasmic reticulum ; this precursor might be the membrane AP endonuclease which is carried by the membrane system, as on a conveyor belt, into the nucleus where the hydrophobic end is cut to free the nuclear sap enzyme ; some of the nuclear sap AP endonuclease might leak into the cytoplasm, but most of it is modified before penetrating the chromatin ; the chromatin enzyme would be the only one important for nuclear DNA repair [14].

Many DNA repair enzymes have been found in rat liver chromatin : uracil DNA-glycosylase, transalkylase, 5'-3' exonuclease, DNA polymerase β, ligase, topoisomerase, etc... and similar activities are found outside chromatin. The synthesis of a precursor which must undergo several steps of maturation before insertion within the chromatin is perhaps not restricted to the AP endonuclease, but might be a general feature for the repair enzymes of nuclear DNA. This hypothesis suggests that repair deficiencies might arise not only from mutations within genes coding for the repair enzymes, but also within genes coding for maturation enzymes or for other chromatin proteins needed for the correct structural arrangement of the repair machinery.

How is DNA containing AP sites repaired in mammalian cells ? Mosbaugh and Linn [17] found in whole extracts of human fibroblasts two AP endonucleases, one cutting 3', the other 5' to the AP site. The successive action of the two enzymes releases the AP site as deoxyribose phosphate ; theoretically, the placement of a single nucleotide followed by ligation is sufficient to complete the repair. We were unable to find in rat liver chromatin an AP endonuclease cutting 3' to the AP site, but rat liver chromatin possesses an exonuclease working on double stranded DNA from nicks in the 5'-3' direction. Could this enzyme excise the AP site after a 5' incision made by the chromatin AP endonuclease ? The answer is yes. DNA containing AP sites was repaired with enzymes prepared from rat liver chromatin : « 0.3 M » AP endonuclease, 5'-3' exonuclease and DNA polymerase β ; although chromatin contains a ligase, in the experiment, ligation was done with T4 ligase [18].

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