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ENZYMES FROM CALF THYMUS THAT MIGHT BE INVOLVED IN DNA REPAIR

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ENZYMES FROM CALF THYMUS THAT MIGHT BE INVOLVED IN DNA REPAIR °

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1. DNA DAMAGE AND REPAIR

The DNA molecules carrying the genetic information of cells are susceptible to damage by radiation of certain wavelengths, such as X-, γ- and UV-rays (see for review: Latarjet, 1972; Blok and Loman, 1974; Murphy, 1974), and by organic chemicals that themselves or through their metabolic products react with nucleic acids as strong electrophiles (Miller and Miller, 1966; Miller, 1970). Living cells exposed to these noxious agents undergo functional inactivation, enhancement of the mutation rate and decrease of survival. In vertebrate animals the lesions of DNA may initiate complex processes of cell transformation that will eventually lead to cancer. Radiation and substances that directly or indirectly alter the molecules of DNA are potentially mutagenic and carcinogenic, thus behaving as genetic toxins. Detrimental agents of this type are natural or artificial components of the environment in which we all presently live.

The DNA cannot be considered as an entirely stable compound. Its primary structure is subjected with time to a number of hydrolytic degradations, even under physiological conditions of temperature and pH. The chemical decay of the heterocyclic base residues in DNA becomes of practical importance for the large genomes of resting and proliferating mammalian cells. Calculations from in

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vitro measurements show that the depurination of dAMP and dGMP nucleotidyl units in the DNA of higher eukaryotes has a definite probability to occur (Lindahl and Nyberg, 1972). Very likely this is equally true for the deaminations of 5-methylcytosine to thymine and also of cytosine to uracil, at least in the dC:dG enriched sequences of the same DNAs (Lindahl and Nyberg, 1974; Lindahl, 1978).

During their lifetime, the cells strive to maintain the functional integrity of genetic material with the aid of serial enzymatic reactions that eliminate incidental lesions from the DNA molecules. These mechanisms of DNA repair are variously distributed in all forms of life and are quantitatively modulated even among the members of a same biological population. The actual capacity to repair DNA is the determinant of the individual sensitivity to the various genetic injuries. Conceivably, DNA repair contributes also to counteract the time dependent accumulation of DNA damages that has been regarded as one of the causes of cellular ageing (Holliday and Tarrant, 1972; Hart, 1976).

Most of our notions about the basic processes of DNA repair come from work carried out in phage-infected or uninfected bacterial cells. Significant progresses were made after the discovery by Ruth Hill of the first radiosensitive mutants in Escherichia coli (Hill, 1958). Thereafter, many bacterial strains with mutations affecting the resistence to radiation and/or to genetic toxins have been isolated. The proper use of crosses between proficient and deficient cells and the genetic analysis of the progeny allowed detection of distinct forms or even single reactions of DNA repair. The informations gained from these genetic experiments greatly facilitated the identification of the competent enzymes. In fact six years after the Hill's report, an integral process of DNA repair, namely the removal of pyrimidine dimers from UV-irradiated DNA in Escherichia coli, could be firstly described as a consistent sequence of enzymatic reactions (Setlow and Carrier, 1964). Up to date other equivalent schemes for the repair of specific DNA injuries in bacteria have been proposed. We shall not elaborate on these studies but we will use the derived conceptual models to outline the general properties and the pattern of the repair mechanisms that are supposed to operate in mammalian cells.

The processes of DNA repair are differentiated and extremely selective in the initial steps brought about by enzymes that recognize only specific molecular damages or unique structures of repair intermediates in DNA. After the removal of altered DNA nulceotides, the repair mechanisms may follow common routes and share biochemical reactions. Conversely, a given DNA lesion can be dealt with by substitutive pathways and a same molecular modification of DNA can be promoted by different enzymes.

It also appears that each system of DNA repair is under the control of numerous genes whose polypeptide products are insufficiently known even for the most investigated Escherichia coli (Clark and Canesan, 1975) and are largely ignored for the highest organisms (Grossman et al., 1975). This applies also to man, as exemplified by the increasing number of mutated genes with unassigned functions that are being recognized in the repair deficient hereditary disorder xeroderma pigmentosum (Bootsma, 1978; Keijzer et al., 1979). It should be reminded that regulatory proteins for transcriptional control of biochemical processes are rare, or perhaps undetectable, in mammalian cells and none of them with a clear relation to DNA repair has been found as yet.

We may reasonably conclude that the mechanisms of DNA repair in vivo are much more intricate and complex than their current description in biochemical terms. Especially for eukaryotic cells, the approach to the biochemistry of DNA repair is mostly restricted to define a minimal sequence of enzymatic reactions which is in agreement with the experimental observations and can coherently explain the corrective changes for restoration of the DNA integrity.

2. DNA REPAIR IN MAMMALIAN CELLS

For a biochemical description, the processes of DNA repair in mammalian cells can be ascribed to three general operations, namely excision repair, post-replication repair and sealing of strand breaks. The first two rely on multi-step mechanisms and the last one is centered around a single enzymatic event. As a complement to these basic pathways, auxiliary reactions promoted by phosphatases, esterases and kinases can provide the altered DNA molecules of the functional termini needed for the attack by repair enzymes.

In this presentation, we shall not consider the photoreactivation of thymine dimers in UV-irradiated DNA, a change mediated either by chemical sensitizers (Roth and Lamola, 1972) or by the enzyme DNA photolyase that has been found also in placental mammals (Sutherland et al., 1974).

2.1. Excision Repair

Excision repair is represented schematically in Figure 1 and refers to a set of transformations in which damaged portions of DNA are eliminated and substituted for by new stretches of the original nucleotides. Through several variants, this mechanism acts on DNA lesions of different origin and with distinct molecular features. In mammalian cells, excision repair has been reported first for UV-induced pyrimidine dimers of human DNA (Regan et al., 1968) and

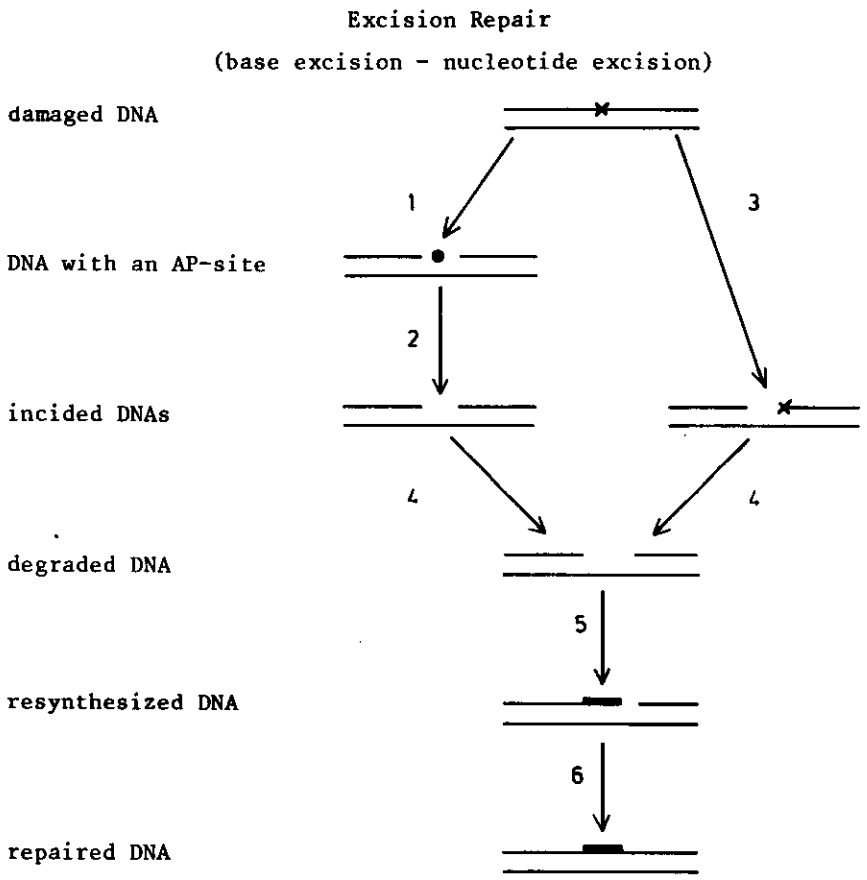

FIGURE 1. Minimal sequence of reactions for excision repair of damaged DNA. The numbered reactions are promoted by various types of enzymes as follows: 1. DNA glycosylases; 2. AP-endonucleases; 3. specific endonucleases; 4. exonucleases; 5. DNA polymerases; 6. DNA ligases.

subsequently for other DNA abnormalities, such as: the 5,6-dihydroxy-dihydrothymine residues formed by ionizing radiation (Mattern et al., 1973); chemically methylated purines (Lawley, 1975); and base adducts produced by a number of carcinogens(Strauss, 1976). The altered nitrogenous bases can be removed from DNA either in the form of nucleotides or as free pyrimidines and purines. These two options have been termed as the nucleotide excision and base excision modes (Lindahl, 1976; see also Hanawalt, 1977).

The overall repair pathway consists of four concerted operations.

1) Incision of DNA near the damaged site. This is regarded as the committed step that is carried out by enzymes recognizing the given DNA lesions. It may occur either in a single endonucleolytic reaction by a specific endonuclease or in a two step process due to the combined action of a DNA glycosylase releasing the altered base and of an apurinic/apyrimidinic endonuclease which cleaves the sugar-phosphate backbone of DNA.

2) Degradation and excision of a nucleotide sequence by an exonuclease that forms a gap in the affected strand of DNA. The size of the gap varies according to the nature of the lesion and ranges from about one hundred nucleotides in UV-irradiated DNA (Cleaver, 1968; Regan et al., 1971) to apparently few base residues in DNA injuries by X-rays (Fox and Fox, 1973).

3) Repair replication by a DNA polymerase that fills the monohelical gap with a new polydeoxynucleotide chain. DNA polymerization starts from the terminal nucleotidyl unit with a free 3'-OH group, at the edge of the gap, and proceeds to complement the unpaired portion of the opposite DNA strand, which is used as a template. After this step a simple monohelical nick with a 3'-OH// 5'-PO_4 conformation is left in the DNA molecule.

4) Sealing of the residual nick by DNA ligase. The enzyme rejoins the contiguous polydeoxynucleotide chains of the interrupted strand and restores DNA integrity.

Excision repair is basically non-mutagenic and error free because it allows accurate correction of structural alterations in DNA. In fact, the defective nucleotide sequence is degraded and resynthesized under the guidance of the intact copy printed in the antiparallel strand of DNA.

2.2. Post-replication Repair

Post-replication repair concerns DNA lesions that escape excision repair. The presence of molecular damages in replicating DNA poses a temporary block to the machinery for DNA synthesis. Nascent polydeoxynucleotide chains stop growing when physical chemical changes are encountered in DNA templates. Gaps in the daughter DNA strands form opposite these lesions and extend up to the next segments of replicated DNA. Afterwards however, DNA polymerization bypasses the damage sites of the parental DNA templates and resumes the assembling of the daughter strands up to completion.

In bacteria, post-replication repair develops with an exchange of DNA material between parental and daughter strands (Rupp et al., 1971) and this sort of illegitimate DNA recombination fills the gaps in the newly synthesized polydeoxynucleotide chains. Recombinational DNA exchanges seem to be infrequent or minimal in the post-replication repair of mammalian DNA (Lehmann, 1972) and alternative mechanisms have been considered (Lehmann, 1975); Higgins et al., 1976). However, the post-replication repair of DNA in mammalian cells has still not been described satisfactorily and the details of its biochemical pathway remain largely to be elucidated. It should be noted that the bypass of DNA lesions during or after DNA replication in mammalian cells share with excision repair both the filling of single stranded gaps by polymerase and the closing of strand breaks by ligase. (Figure 2).

The replication of DNA containing molecular alterations may result in the formation of incorrect nucleotide sequences. Therefore, post-replication repair has been viewed mainly as error prone, although some of the proposed mechanisms for such a repair imply faithful duplication of the DNA strands (Higgins et al., 1976).

It has been suggested that a mutagenic post-replicative DNA repair, called "SOS repair", can be induced in prokaryotes by agents causing DNA lesions (Radman, 1974). The presence of a similar inducible error prone process of DNA repair in mammalian cells has not been proved conclusively.

2.3. Sealing of Strand Breaks

Breaks in the sugar-phosphate backbone of DNA strands are produced with a high incidence by ionizing radiations (see: Latarjet, 1972; Blok and Loman, 1973), but they are also found after heavy exposures to UV-light (Marmur et al., 1961) and treatment with metal-binding antibiotics of the bleomycin type (Taheshita et al., 1978). The nicks formed in DNA are not always hydrolytic scissions of the phosphodiester bridges between the deoxynucleotides. Often, they are associated with chemical changes of the deoxyribose resi-

Post-replication Repair

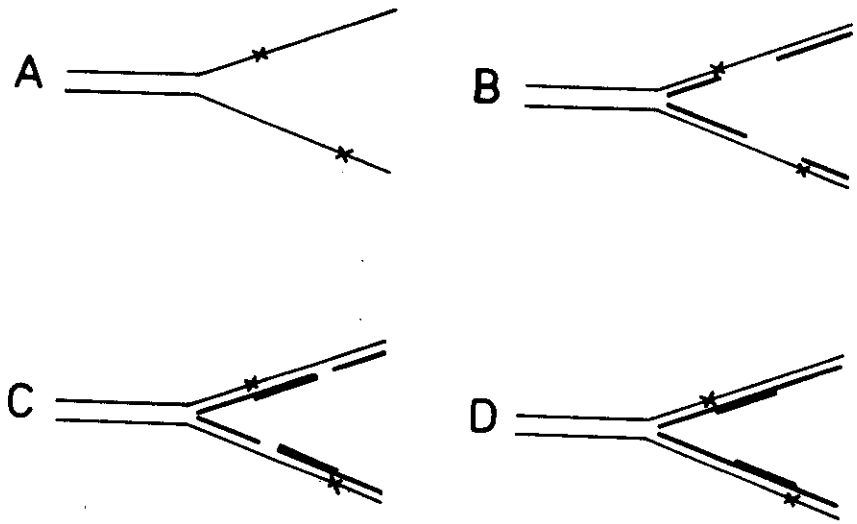

FIGURE 2. Scheme of events in post-replication repair of mammalian DNA.
A. Opened replication fork with DNA damaged
B. Replicated fork with gaps in daughter strands
C. DNA polymerization bypassing lesions
D. Ligation of newly formed DNA chains.

dues, release of the bases and even loss of terminal nucleotidyl units. In the last instances, the sealing of strand breaks is coupled with reactions of the excision repair pathway.

The enzyme DNA ligase is able to close directly the single stranded breaks of DNA that have a 3'-OH//5'-PO_4 conformation. For other simple monohelical interruptions, the 3' and/or 5' chemical termini in the nicks must be properly modified by auxiliary enzymatic reaction before ligation takes place.

The rejoining enzyme shows an absolute specificity for justaposed 3'-OH and 5'-PO_4 termini in the sealable nicks, but it has a loose requirement for the integrity of the nucleotide sequences in the broken DNA chains. Sealing of mohohelical scissions in DNA is a non-limiting process for mammalian cells and occurs also after supra-lethal doses of ionizing rays (Lett et al., 1967). Surely, much of the rejoining in the irradiated cells must have been carried out on damaged DNA and has to be ascribed to misrepair.

The ligase property to act also on DNA molecules with structural alterations is of practical importance. In fact, ligation of an interrupted DNA strand still endowed with nucleotide lesions might well facilitate the fixation of premutational changes in the genetic material. Since the closing of a DNA nick is the last step of functional DNA repair, mechanisms for elimination of base damage from DNA in a concerted manner with the sealing of strand breaks should operate in vivo. However, the control of DNA repair as to prevent untimely ligations has not been studied in animal organisms.

The reported rejoining of double stranded scissions in mammalian DNA (Corry and Cole, 1973) may reflect the relaxed requirement of ligase for the spatial structure of DNA substrates. Occasional blunt joining of contiguous bihelical DNA fragments will appear as a repair of double stranded breaks and this may well occur for the folded DNA molecules of nuclear chromatin. However, the relevance of such processes for reconstitution of a functional genome and/or induction of chromosomal aberrations remains to be established.

3. ENZYMES OF DNA REPAIR FROM CALF THYMUS

Bovine thymus represents a sort of model tissue for the study of mammalian enzymes that act on DNA substrates and DNA templates. In fact, it is the single mammalian source from which the largest number of these functional proteins have been prepared and characterized. A short survey of the calf thymus enzymes that may have a role in DNA repair pathways, here conceived as minimal sequences of possible biochemical reactions, will provide a useful framework of knowledge.

The four main classes of enzymes that have been implicated in the repair of mammalian DNA, namely DNA glycosylases, DNases, DNA polymerases and DNA ligases, are all present in calf thymus and enzymes of each class have been the target of detailed investigations.

3.1. DNA Glycosylases

These glycosylases remove directly damaged or incorrect bases from DNA and are the first components of base excision systems for DNA repair. Each enzyme has a narrow specificity for a given abnormal base and cleaves the sugar-base glycosyl linkage of an improper deoxynucleotidyl unit in DNA, thus releasing the base and leaving an apurinic-apyrimidinic site. This site is recognized by a specific DNase, AP-endonuclease, which hydrolyzes its phosphodiester bond and produces a single strand break with partial or total loss of a nucleotidyl residue. After the strand scission, the structural integrity of DNA might be recovered through the series of exonuclease, DNA polymerase and DNA ligase reactions occurring after the initial incision step in the excision repair process.

The uracil-DNA glycosylase from calf thymus has been studied in our laboratory. The enzyme was partially purified from extracts of thymocyte nuclei and characterized. It catalyzes the release of uracil from synthetic DNAs containing uracil residues, uracil-DNAs, and has an estimated molecular weight of 28,700. The glycosylase reaction does not require cofactors nor divalent metal cations, occur on polymeric substrates and not on dUMP, and is clearly of the hydrolytic type with an equilibrium greatly shifted towards the dissociation of uracil from DNA (Talpaert-Borlé et al., 1979). With the exception of the preference for double stranded uracil-DNAs as substrates and of the higher molecular weight, the enzyme properties are similar to those reported for the analogous bacterial enzymes by other investigators (Cone et al., 1977; Lindhal et al., 1977).

The obvious role of this glycosylase is to remove from DNA the uracil which is formed by deamination of cytosine or in incorporated by polymerization of dUTP instead of dTTP substrates. The enzyme is rather ubiquitous and has been obtained from a number of animal sources. We have detected uracil-DNA glycosylase activity in extracts from the main parenchimatous tissues of rat, the highest concentration being found in thymus (Talpaet-Borlé and Campagnari, unpublished results).

Other DNA glycosylases for base excision have not been carefully investigated in mammalian cells. Although some of the reported reactions might need better identification, circumstantial evidence

for glycosylases active on alkyl-DNAs has been forwarded (Lawley, 1975). An enzyme releasing 3-methyladenine from alkylated DNA has been recovered from cultured human lymphoblasts (Brent, 1979). This glycosylase does not require divalent metal cations and has an estimated molecular weight of 34,000. Therefore, we may consider that at least one alkyl-DNA glycosylase, namely the enzyme acting on 3-methyladenine-DNA, is functionally expressed in mammalian lymphoid tissues, including thymus.

3.2. AP-endonuclease

An enzyme which introduces single strand breaks at the apurinic-apyrimidine sites of bihelical DNA has been isolated from calf thymus. It has a molecular weight in the 30,000-35,000 range and displays optimal catalysis at pH 8.5 and in the presence of Mg^{++} or Mn^{++} (Ljungquist et al., 1974a). The AP-endonuclease cleaves DNA at the 3' side of the bare deoxyribose residue with the release of a 5'-PO_4 nucleotide terminus. It is active on heat depurinated DNA and on irradiated DNA with alkali labile sites that correspond to base losses in single nucleotidyl units (Ljungquist et al., 1974b).

This enzyme is a monofunctional protein without associated phosphatase and exonuclease activities and can be considered as representative of mammalian AP-endonucleases. It is at variance with the main AP-endonuclease of bacterial cells, namely endonuclease II, which performs multiple catalytic functions and should be better termed as exonuclease III* (Weiss, 1976).

3.3. Endonucleases

Early reports indicate that a DNase activity of type II is present in nuclei of calf thymus cells (Slor and Lev, 1971). This enzyme would be similar to the lysosomal acid DNase which degrades DNA to oligonucleotides by promoting hydrolytic chain scissions with a 3'-PO_4//5'-OH conformation (Bernardi, 1971). The nuclear form of calf thymus DNase II has not been clearly identified and its function, especially with regard to DNA repair, is rather dubious.

Another endonuclease, that is also detectable in cell nuclei but might well be involved in DNA repair, has been purified to homogeneity from bovine thymus and has been distinctly characterized (Wang and Furth, 1977). It is a tetrameric protein composed of identical subunits with molecular weights of 13,000 and possessing an exceptionally high isoelectric point. The enzyme shows a pH optimum at 6.6 and a requirement for Mg^{++} or Mn^{++}; it hydrolyzes

extensively single stranded DNA but introduces only a limited number of monohelical nicks into native DNA. It is unknown whether this limited attack on DNA reflects a specificity for rare base sequences or for casual changes in the structure of double stranded DNA. Apparently, this endonuclease has several properties that are expected attributes of a DNA inciding enzyme for repair. Such an enzyme has been named DNase V and differs from the pancreatic DNase I, which also forms 3'-PO_4 strand breaks but degrades native DNA almost to completion (Laskowsky, 1971).

An example of mammalian endonuclease specific for DNA lesions is provided by another calf thymus DNase, which has been partially purified and found to hydrolyze internal phosphodiester bonds in irradiated double stranded DNAs (Bacchetti and Benne, 1975). This enzyme is active also in the absence of divalent cations, recognizes apparently unidentified chemical alteration caused in DNA by either UV-light or γ-rays, and releases 5'-PO_4 termini at the incision sites.

Many of the endonucleases for DNA repair that have been noted in various mammalian tissues can be safely assigned to the DNase types isolated from calf thymus.

3.4. Exonucleases

After the enzymatic incision of damaged DNA, the defective nucleotidyl residues in or nearby the strand break should be removed by exonucleases. These enzymes have not been investigated in calf thymus glands as the endonucleases. However, two exonucleases, namely DNase III and DNase IV, have been isolated from rabbit bone marrow by Lindhal and coworkers and will be shortly described. Both are Mg^{++}-dependent nucleases localized in cell nuclei.

DNase III has an estimated molecular weight of 52,000 and hydrolyzes sequentially polydeoxynucleotide chains from the 3' ends thus liberating 5'-mononucleotides and dinucleotides. Although it has a preference for single stranded DNA as substrate, it degrades at appreciable rates also native DNA (Lindhal et al., 1969a).

DNase IV has an approximate molecular weight of 42,000 and promotes exonucleolytic degradation of double stranded DNA from the 5' ends with release of 5-mononucleotides. The enzyme appears to be specific for bihelical DNA substrates and hydrolyzes the first nucleotides in DNA at higher rates than the subsequent ones (Lindhal et al., 1969b).

Exonucleases similar to DNase III and DNase IV are widely distributed and can be detected as contaminants of other enzyme

preparations from nuclei of mammalian cells. As a matter of fact, we reported the presence of DNase III activity in side fractions obtained during the purification of a DNA polymerase from the nuclei of calf thymocytes (Bekkering-Kuylaars and Campagnari, 1974).

It should be mentioned that a 3' → 5' exonuclease with "proof-reading" function for DNA synthesis has been found associated with DNA polymerase α in partially purified preparations from nuclei of calf thymus cells. This 3' → 5' exonuclease excises in vitro mismatched nucleotides from the 3'-OH termini of synthetic DNA primers in a coordinate manner with the DNA polymerase reaction; such an activity is lost in the advanced stages of purification when the polymerase seems to separate into low molecular weight polypeptides (Clerici et al., 1980).

It is feasible that exonucleases present in cell nulcei and active on double stranded DNA, such as DNase III, DNase IV and the "proofreading" enzyme, exert repair functions on th genetic material in specific occurrences.

3.5. DNA polymerases

The two main DNA polymerases found in calf thymus glands, namely polymerase α and polymerase β, have been largely characterized by Bollum and coworkers and the gained knowledge has inspired most of the subsequent work on the DNA synthesizing enzymes from mammalian cells (see: Bollum F.J., 1975).

The DNA polymerase α of bovine thymus has been originally purified and is usually recovered from cytoplasmic fractions (Yoneda and Bollum, 1965; Bollum et al., 1974), but it is present in appreciable quantities also in cell nuclei from which has been extracted by mild procedures and purified (Bekkering-Kuylaars and Campagnari, 1972). The enzyme is heterogeneous and has been obtained in a number of forms with molecular weights ranging from over 230,000 to about 130,000 (Momparler et al., 1973; Bollum et al., 1974; Holmes et al., 1976). This reflects the breaking down of either an enzyme aggregate or a functional complex during isolation. Extensive purification of both cytoplasmic and nuclear enzymes gives rise to unstable or inactive forms with molecular weights well below 100,000 (Bollum et al., 1974; Yoshida et al., 1974; Bekkering-Kuylaars and Campagnari, 1974; Holmes et al., 1979). A polypeptide or enzyme fragment with molecular weight of 70,000 has been indicated as the active subunit common to all species of DNA polymerase α (Bollum et al., 1974; Bollum, 1975).

In the presence of dNTP substrates, the polymerase α promotes DNA synthesis on various primer template systems, such as self-

primed single stranded DNA, native DNA with monohelical gaps and synthetic DNA templates paired with either deoxyribonucleotide or ribonucleotide primers. Its ability to utilize RNA chains as primers has been related to a role in initiating DNA replication in vivo (Chang and Bollum, 1972). In fact, this enzymatic activity is always high in tissues with many dividing cells, like thymus, and has been found to increase in response to stimuli for cell proliferation (Bollum, 1975).

The α-polymerase shows pH optimum for activity at neutrality, requires Mg^{++} or Mn^{++} as metal cofactors respectively with DNA or homopolydeoxynucleotide templates, and is inhibited by concentrations of 0.1 M salts and of 1 mM N-ethylmaleimide. The enzyme catalysis on the primer-templates occurs in a distributive manner through short rounds of nucleotide polymerization and not by formation of a long DNA chain at a single time (Chang, 1975). Only a trace level of 3' → 5' "proofreading" exonuclease activity has been detected in the calf thymus DNA polymerase isolated from cytoplasmic fractions, whereas an error correcting exonuclease seems to be present in partially purified preparations of the DNA polymerase α extracted from cell nuclei as already mentioned (Clerici et al., 1980).

With regard to the enzyme activity on damaged DNA, it appears that the complementary duplication of the template chain is induced at normal rates on mildly X-irradiated DNA (Campagnari et al., 1967) but is blocked and does not go to completion on DNAs exposed to heavy doses of UV-light (Bollum and Setlow, 1963) or to X-ray doses over 2,000 and up to 10,000 rads (Campagnari and Bertazzoni, 1968).

The DNA polymerase β of calf thymus is mainly bound to the nuclear chromatin, from which it has been purified to homogeneity, and is detectable also in cytoplasmic fractions. The enzyme consists of a basic polypeptide with a molecular weight of 45,000 and acts in the presence of suitable templates, complementary dNTPs and Mg^{++} or Mn^{++} as divalent metals. Its catalytic properties are similar with those of the α-polymerase, but it has a rather alkaline pH optimum, is insensitive to N-ethylmaleimide and is stimulated, not inhibited by 0.1-0.2 M salts (Chang, 1973a). Moreover at variance with the enzyme α, this deoxynucleotidyl-transferase induces DNA synthesis only by elongating DNA primers (Chang and Bollum, 1972), while being able to use effectively RNA chains as templates (Bekkering-Kuylaars and Campagnari, 1974; Bollum, 1975). Although the purified DNA polymerase β does not have an associated 3' → 5' exonuclease with error correcting function, it has been found to replicate complementarily the templates with high accuracy and with a mistake frequency below 10^{-4} (Chang, 1973b).

The amount of this enzyme in bovine thymus is low and falls

within the narrow range of values observed in other mammalian organs in terms of units/g of tissue. The DNA polymerase is present in all cells at almost the same constant concentration, quite independently upon metabolic changes and proliferative states (Bollum, 1975). The properties and the distribution of this enzyme are suggestive evidence for its role in DNA repair. However, it is unknown whether DNA repair synthesis can be alternatively or substitutively carried out also by the polymerase α in the late G_1, S and early G_2 phases of the cell cycle, when high levels of this DNA replicative enzyme are available.

3.6. DNA ligase

Like other eukaryotic ligases, the DNA-joining enzyme partially purified from calf thymus uses ATP-Mg as an energy donor to seal single strand breaks in native DNA and has an absolute requirement for the presence of adjacent 3'-OH and 5'-PO_4 termini at the nicks (Bertazzoni et al., 1972).

As already anticipated, mammalian ligases are rather unspecific for the correct structure of DNA outside the internucleotide scission. In fact, the calf thymus enzyme has been found capable to rejoin breaks in natural and synthetic DNAs, previously exposed to X-rays and thus containing significant radiation damage (Mathelet et al., 1974). In these experiments, all the nicks with the expected 3'-OH//5'-PO_4 conformation were sealed by ligase, although the alterations in the nucleotides close to the breaks prevented some specific marker enzymes from recognizing the functional 3'-OH termini of the broken DNA chains. It is well possible that misrepair by ligation is more frequent in mammalian cells than in bacteria, which possess a NAD^+-dependent ligase with more stringent catalytic requirements (Lehman, 1974).

The above observations have been made with a ligase purified about 3000-fold over the thymus homogenate and corresponding to the main enzyme form which has been indicated as DNA ligase I. According to data from various mammalian tissue, this is a large enzyme composed of various subunits and increasing in cells during DNA replication (Soderhall and Lindhal, 1976).

The significance of the minor enzyme form called DNA ligase II, which is more labile and has a lower molecular weight than DNA ligase I, is still doubtful. This enzyme has been extracted also from calf thymus, where it represents less than 7% of the total ligase activity, and has been found not to react with antisera prepared against purified DNA ligage I (Söderhäll and Lindhal, 1975).

4. CONCLUDING REMARKS

An inventory of the enzymes isolated from calf thymus and shown to act on different DNA structures has been performed with reference to the general schemes of DNA repair in mammalian cells. The survey has been forcibly restricted to the few ascertained reactions occurring in vitro on DNA molecules dispersed in aqueous solutions. Therefore, the direct attack of enzymes on DNA packaged with histones and nuclear proteins in the chromatin has not been considered, although this functional interaction determines the success or the failure of the DNA repair events in the cell nuclei (Smerdon et al., 1978). Moreover, the absence of specific informations for the thymus tissue has given rise to other omissions. In fact, the repair of mitochondrial DNA and the newly discovered enzymatic activities, such as that of the putative purine-DNA insertase isolated from human cells (Deutsch and Linn, 1979), have not been taken into account.

Nevertheless, the exercise of confronting established enzyme properties with potential functions in DNA repair might have been useful. The following conclusions can be warranted.

a) For mammalian DNA repair, a wide gap exists between the scarce notions on the enzymes involved and the large body of evidence for the numerous and complex pathways that operate in cells (see for review: Cleaver, 1978). A comprehensive biochemical description of DNA repair in mammalian cells is still premature, the only reasonable approach being the attempt to define minimal sequences of repair reactions. At the present time, the main contributions in this area by biochemists may well come from the work directed to characterize the action of single enzymes on DNA integrated into chromatin and to identify new types of enzymatic activities.

b) The enzymes of DNA repair perform vital functions and are indispensable. Their concentration in mammalian cells fluctuate within narrow ranges of values with the exception of the increases noted during the proliferative state for those activities that are involved also in DNA replication. It is unlikely that mammalian zygote, not endowed with a full complement of DNA repair reactions, can develop through the stages of embryonic and fetal life and will give rise to a viable organism.

Therefore, we may expect to find rarely humans that are born with truly enzymatic defects of DNA repair. This is much at variance with the frequent enzymatic etiology of many inborn errors of metabolism. Generally, in fact, the relevant enzyme activities are at almost normal levels in the cells from the inherited human diseases

with defective process of DNA repair. So far the only genetic deficiency of a repair enzyme might have been detected in a patient with xeroderma pigmentosum of complementation group D, whose cells had one-sixth of the activity normally displayed by an Ap-endonuclease (Kuhnlein et al., 1976).

c) The enzymes acting on DNA are essential component of the mechanisms for DNA repair but are not the limiting factors in the functional operating of such mechanisms inside mammalian cells. Non-enzymatic gene products controlling or facilitating the biochemical reactions on nuclear chromatin are probably responsible for most of the inborn defects of DNA repair in man.

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ERRATA CORRIGE

pg. 140,	line 4:	nulceo	nucleo
pg. 147,	line 25:	occur	occurs
pg. 147,	line 34:	or in	or is
pg. 149,	line 8:	3'-PO_4	5'-PO_4
pg. 155,	line 37:	pp 23-30	pp 27-34