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NUCLEOTIDE EXCISION REPAIR, TRANSCRIPTION AND HUMAN REPAIR SYNDROMES

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DNA repair systems prevent the effects of damage induced by genotoxic agents such as genetic instability and cancer. Inherited defects in the nucleotide excision repair (NER) system in xeroderma pigmentosum (XP), Cockayne syndrome (CS) and PIBIDS are characterized by sun-(UV) sensitivity, predisposition to skin cancer (in XP) and a remarkable clinical and genetic heterogeneity. Recent analysis of several NER genes has revealed that some have an additional function in transcription or recombination. This may account for part of the unexpected clinical symptoms in CS and PIBIDS such as brittle hair, poor growth and neurodysmyelination. Strong sequence homology between human and yeast NER proteins indicates that the NER pathway is highly conserved in eukaryotic evolution. The development of an in vitro NER assay and the isolation of NER genes has permitted partial dissection of the complex reaction mechanism. Finally, the use of gene targeting in totipotent embryonal stem cells has enabled the generation of mouse models for human repair disorders which are expected to be of great value for cancer research and genotoxicity assays in general.

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REPAIR OF OXIDATIVE DNA DAMAGE: ROLE OF THE HAP1 PROTEIN.

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Reactive oxygen species mediate most of the cytotoxic effects of ionizing radiation and radiomimetic drugs such as bleomycin. These species damage DNA producing a wide range of lesions which can be both toxic and mutagenic. Damage to DNA is repaired by a battery of DNA repair enzymes one of which, the HAP1 protein, we have been studying. The HAP1 protein is the major enzyme for repair of apurinic/apyrimidinic (AP) sites induced by reactive oxygen species and participates in the repair of DNA strand breaks with 3' terminal fragmented sugar derivatives which are a characteristic lesion produced by x-rays and bleomycin. We have cloned the HAP1 gene and isolated cDNA clones encoding HAP1 protein. This has permitted overexpression of the HAP1 protein in milligram quantities in E. coli for structural and biochemical analyses. The HAP1 protein has also been identified as a protein redox state modifier which can reduce and consequently activate oxidized transcription factors such as c-jun and c-myb. Using site directed mutagenesis, we have been able to separate the DNA repair and redox functions of the HAP1 protein and have shown that these functions lie within different domains of the protein.

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ROLE OF POLY ADP-RIBOSYLATION IN DNA REPAIR

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The introduction of DNA strand breaks in the genome of eukaryotic cells that have been exposed to radiations or genotoxic agents, triggers the endogenous synthesis of poly (ADP-ribose). The poly (ADP-ribose) polymerase (PARP) responsible for this post translational modification catalyses the transfer of the ADP-ribose moiety from the substrate NAD, to several chromosomal acceptors proteins during repair. In order to understand the molecular basis of this complex mechanism we have dissected and studied the different functional domains of the human enzyme. In particular, the DNA binding domain (DBD) containing both, two zinc fingers involved in DNA strand break detection and a nuclear localisation signal, has been overproduced in CV-1 cells or microinjected into normal human fibroblasts giving a trans-dominant negative phenotype. Under these conditions the resident PARP activity is blocked and in turn, DNA repair synthesis induced by an alkylating agent is totally inhibited. No inhibition was observed after UV (254 nm) irradiation. PARP thus appears as a critical regulatory component inthe repair of DNA damage induced by alkylating agents.

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BASE EXCISION DNA REPAIR

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The base excision repair pathway plays an important role in the repair of DNA damage caused by spontaneous hydrolytic decay of DNA i.e. depurination and base deamination. This form of DNA repair is also involved in the cellular defence against simple alkylating agents and oxygen free radicals. Double-stranded oligodeoxyribonucleotides containing a single uracil residue at a defined position were employed as substrates to study the molecular mechanisms of base excision repair. The uracil-containing oligonucleotides were efficiently repaired by gently prepared cell extracts from *E.coli* and HeLa cells. Restriction enzyme analysis of the corrected oligonucleotides showed that cell extracts from both sources preferentially carry out repair by replacing a single nucleotide. The events resulting in replacement of a single damaged nucleotide residue were reconstructed *in vitro* with purified *E.coli* enzymes.

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INVOLVEMENT OF DNA TOPOISOMERASES IN RECOMBINATION AND DNA REPAIR.

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In most biological systems DNA is topologically constrained due to intertwining of the two DNA strands. This property of DNA allows for higher order structures, which can dictate essential biological functions. Thus, unwinding facilitates processes such as DNA replication, transcription, and recombination. Regulation of DNA topology is therefore of critical importance to normal cellular functions. In eukaryotic cells the topological structure of DNA is modulated by the enzymes called topoisomerases. These enzymes can be divided into two classes, type I and type II, which both interconvert different topological forms of DNA by creating transient breaks in the DNA backbone. Beyond their normal physiological functions, topoisomerases are important cellular targets in the treatment of human cancers. Considerable effort has therefore been invested to clarify the molecular mechanism of interaction between the drugs and their enzyme target. We present at the meeting a set of new techniques which have allowed us to study the involvement of DNA topoisomerases in different types of recombination and repair.

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DNA REPAIR DEFECTS AND CANCER PRONENESS.

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Specific defects in one of the processes operating in the cell to repair DNA damage have been implicated in a number of autosomal recessive disorders. These diseases include xeroderma pigmentosum, ataxia telangiectasia, Fanconi's anemia and Bloom's syndrome. In addition to alterations of immunological and neurological functions, and of cellular parameters such as chromosomal stability, viability and mutability, the impaired DNA repair capacity in these disorders is coupled with an increased incidence of tumours. Despite their rare occurrence, these genetic defects are of general importance for the high cancer susceptibility seen in heterozygous carriers who are a significant proportion of the population.
However, there are two other disorders (trichothiodystrophy and Cockayne's syndrome) that do not show any association with cancer proneness, although they are defective in DNA repair. Therefore the definition of the functions altered in these diseases will hopefully clarify some crucial steps of tumoral transformation and progression.