DNA Repair

S13-01

XPG, a DNA repair protein implicated in two human diseases
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The human gene *XPG* (also known as *ERCC5*) encodes a structure-specific endonuclease of 1186 amino acids that nicks the damaged DNA strand 3' to the lesion during an early phase of nucleotide excision repair (NER). When defective, this gene can give rise to two autosomal recessive disorders, xeroderma pigmentosum (XP) and Cockayne syndrome (CS). XP patients are hypersensitive to sunlight, most have a predisposition to skin cancer, and some show severe neurological abnormalities. CS is characterized by dwarfism, severe mental and physical retardation, gait defects and acute sun sensitivity but no cancer predisposition. I shall discuss recent clues as to how defects in the *XPG* gene can give rise to these two very different clinical phenotypes, and the potential implications of these clues for a second important XPG function.

S13-02

SEQUENCE-SPECIFIC AND DOMAIN-SPECIFIC DNA REPAIR IN HUMAN CELLS
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Sensitive techniques are now available to study repair of DNA damage at high resolution. Repair of UV-induced cyclobutane pyrimidine dimers (CPDs) is DNA sequence-dependent. In the p53 gene, slow repair of CPDs correlates with mutational hotspot positions in nonmelanoma skin cancers. Recently, we observed a new form of DNA repair heterogeneity. Repair of CPDs was measured along the human *JUN* gene in diploid fibroblasts. Sequences of the upstream promoter are repaired poorly. Repair rates are 10-times faster on both DNA strands near the transcription initiation site. Such fast repair may be explained by increased local concentrations of DNA repair factors associated with general transcription factors (e.g. TFIIH) functioning in transcription initiation. This domain-specific DNA repair may aid in maintaining transcription initiation of essential genes after DNA damage.

S13-03

DNA Damage and Repair Processing in Chromatin
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The location and longevity of UV damaged sites in DNA is modulated by the packaging of DNA in chromatin. At the nucleosome level, the major UV photo product (CPD) forms a characteristic 10.3 base repeat pattern, due to bending of DNA around histones. Conversely, (6-4) dimers [(6-4)PD] form randomly in nucleosome cores with a strong bias toward "nuclease sensitive" regions in chromatin. Repair of (6-4)PDs in nucleosome cores is very rapid, however, and the rapid repair of (6-4)PDs observed in the genome overall does not reflect this distribution.

Transcriptionally active chromatin is repaired preferentially due to efficient removal of damage from the transcribed strand of active RNA polymerase II genes. Determining if multicopied ribosomal genes (rDNA), transcribed by RNA polymerase I, are repaired preferentially or strand specifically is more complex because only a fraction of these genes is transcriptionally active. Selective psoralen binding was used to separate the active fraction from inactive rDNA, and *Eco*RI digestion was used to selectively release the active fraction from nuclei. There was no evidence for efficient repair of CPDs or "transcription repair coupling" in the individual strands of active (or total) rDNA. Even the first phase of nucleotide excision repair (chromatin rearrangement) does not appear to occur in rDNA. However, bleomycin induced strand breaks are repaired efficiently in ribosomal genes, indicating that this mechanism has "access" to ribosomal chromatin.

S13-04

Repair of Cyclobutane Pyrimidine Dimers by Photolyase in Yeast Chromatin
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We analyzed the effect of chromatin structure on the repair of cyclobutane pyrimidine dimers (CPDs) by photolyase in yeast minichromosomes with known chromatin structures. The *S. cerevisiae* strains JMY1 (rad1Δ) and FTY117 (rad1Δ), which are deficient in nucleotide excision repair, were irradiated with UV light (254 nm) at a dose of 100 J/m^2. For repair, cells were irradiated with photoreactivating light (366 nm). DNA was extracted and digested with restriction enzymes. DNA was cut at CPDs with T4-endoV and the cutting sites were displayed by indirect endlabelling, using strand specific RNA probes. The results show fast removal of CPDs in regions which strictly correlate to nuclease sensitive 'open' regions, whereas DNA which is known to be packed into nucleosomes is repaired more slowly.

S13-05

DIFFERENTIAL RECOGNITION OF BULKY DNA ADDUCTS BY HUMAN NUCLEOTIDE EXCISION REPAIR (NER)
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Mammalian NER processes a wide spectrum of bulky carcinogen-DNA adducts, but the mechanism by which this system discriminates between damaged and undamaged DNA is poorly understood. We set up an in vitro competition assay to assess recognition by human NER in a quantitative manner. This assay is based on a site-directed NER substrate, and measures the efficiency by which damaged plasmids inhibit excision repair of the substrate by competing for repair factors. Using this novel recognition assay, we observed enormous (>300-fold) differences in the ability of human NER to sense structurally distinct carcinogen-DNA adducts. This recognition capacity decreases with the following order of bulky adducts: AAF > UV photoproducts > benzo[*a*]pyrene diol epoxide > anthramycin, CC-1065. Our results indicate that gross helical deformity is not absolutely required for recognition by the human NER system. However, the observed substrate preferences indicate that abnormal exposure of aromatic residues on the DNA surface may constitute a molecular signal for the recruitment of recognition factors at sites of damage.

S13-06

PHYSICAL HETERODUPLEX DETECTION IN THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*
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In this study, we use denaturant gel electrophoresis to physically detect heteroduplex DNA formed during meiotic recombination. We analyze heteroduplex formation at *ura4-A6*, a G to C transversion mutation in the recombination hotspot *ura4-aim* (aim: artificially introduced marker between *ade6* and centromere III). The high level of postmeiotic segregation (PMS) of this mutation enables mismatch detection. Our results indicate that the G·G mismatch is repaired efficiently and thus not detected in late stages of meiosis. C·C escapes correction and is therefore detectable at the end of meiosis. In contrast to wild type, strains with mutations in either of two mismatch repair genes (*mlh1* or *msh2*) show (besides C·C) also G·G heteroduplex molecules. These findings show, that both genes investigated are really involved in mismatch repair. The exact timing of heteroduplex formation relative to other events in meiosis is currently being studied in more detail.

S13-07

CHROMOSOME BREAKAGE AND HEALING IN *ASCARIS SUUM*: A DEVELOPMENTALLY PROGRAMMED REARRANGEMENT DURING CHROMATIN DIMINUTION

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In the early embryonic development of the parasitic nematode *A. suum*, the chromosomes undergo chromatin diminution in all presomatic cells. During this process, which occurs between the third and the fifth embryonic cleavage divisions in five different blastomeres, the heterochromatin of the chromosomes is removed and becomes degraded. The chromosomes are healed by the de novo addition of telomeric repeats. New telomere addition takes place within a well-defined region, called CBR (chromosomal breakage region), of which many copies are present in the *Ascaris* genome.
We have analyzed and compared the structure of three cloned CBRs (CBR1, 2 and 3). Southern blots and PCR experiments on somatic DNA revealed that addition of the newly-formed telomeres occurred randomly at many different sites within the CBRs. No sequence or secondary structure homologies are shared between the different telomere addition sites. The presence of 1-4 ambiguous nucleotides at the junction between the CBR sequences and the newly added telomeric repeats strongly suggests that the healing of the truncated chromosomes is mediated by telomerase activity (see Magnenat *et al.*). Surprisingly, telomeres are not only added to the ends of the truncated somatic chromosomes, but also to the ends of the eliminated chromatin before it becomes degraded.
We are developing a biological test system to determine the role of the CBR sequences and are investigating the importance of chromatin structure in the CBRs before, during and after elimination.

S13-08

In vitro V(D)J recombination in extracts from a murine B-cell line overexpressing the rag-1 and rag-2 proteins.

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The rag-1 and rag-2 proteins have been shown to cleave DNA at the V(D)J specific recombination signal sequences (RSS). Here we show that extracts from a B-cell line overexpressing truncated forms of both rag proteins contain all the necessary enzymatic acitvities for RSS specific cleavage and the generation of V(D)J specific joining products. A plasmid recombination substrate containing a pair of RSS was incubated with cellular extract. The recovered substrate was found to be cleaved to a significant extent, and products characteristic for coding joints (CJ) were obtained by PCR. Cloning and sequencing of these products revealed V(D)J characteristic CJ with deletions and P nucleotides. This in vitro V(D)J recombination system should allow a more detailed study of the enzymatic activities involved.

S13-09

DNA mismatch repair in the fission yeast *S. pombe*: Investigation of the *mutLS* like pathway

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To investigate DNA mismatch repair by the *mutLS* like pathway in *S. pombe*, we have cloned a *mutS* and a *mutL* homolog, designated *msh2* and *mlh1* respectively. Genetic analyses indicate, that *mlh1* and *msh2* are both involved in mismatch repair. Surprisingly *msh2*, but not *mlh1* also has a function in mating-type switching. In addition, the general mismatch binding activity is absent in the *msh2* gene disruption.

S13-10

CHARACTERIZATION OF MISMATCH-BINDING PROTEINS OF *SCHIZOSACCHAROMYCES POMBE*

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We have identified two mismatch-specific binding activities by band shifts. The general binding activity seems to be absent in the *swi8-137* (*=msh2*) mutant, while the C/C binding activity is present in all tested mutants.

We have started the purification of the C/C binding activity. A partial purified fraction containing both activities was tested for binding to DNA-loops with 2 or 4 unpaired cytosines or thymidines. While the general mismatch-binding activity efficiently binds to DNA-loops, the C/C binding activity shows only weak affinity to this type of mismatches.

S13-11

NEW PROTEINS INTERACTING WITH THE *S.CEREVISIAE* RECOMBINATIONAL REPAIR PROTEIN RAD54

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The *RAD54* gene of *S. cerevisiae* is a central function in recombinational repair. Mutations in this gene lead to extreme sensitivity to ionizing radiation, to a slight UV-sensitivity and to defects in genetic recombination. Recombinational repair is thought to be carried out by a multi-protein complex. Therefore, we have been using two molecular-genetical approaches (two-hybrid system and high-copy suppression of dominant negative *RAD54* alleles) to potentially identify proteins that interact with Rad54. So far two candidates have been found. Haploid cells deleted for these genes exhibit DNA damage repair phenotypes providing evidence for the biological significance of the observed interaction. Besides the genetic and phenotypic characterization of these novel genes we are carrying out experiments to identify Rad54 containing complexes *in vivo* and to demonstrate direct interaction of the proteins *in vitro*.

S13-12

EPISTATIC GROUPS & DNA REPAIR GENES IN ASPERGILLUS

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In *A. nidulans*, four epistatic Uvs groups were identified by genetic tests. Recent results from gene cloning and sequence homologies with yeast and mammalian genes indicate possible functions: 1) UvsF group genes code for proteins active in an error-free post-replication repair; 2) UvsC group genes are required for mutagenic and recombinational repair (similar to *E. coli recA*); 3) *uvsI* acts in a base-pair-specific type of mutagenic repair; 4) UvsB group genes may function in ds-break repair, possibly by end-joining. Therefore no genes active in nucleotide excision repair have been found. While mutants of the *uvsF* gene show the expected phenotypes (normal growth and meiosis, increased UV-induced mutation and spontaneous mitotic recombination), we found that *uvsF* does not code for an excision protein, but shows sequence homology to the DNA replication factor RFC1, active in DNA repair.

S13-13

Saccharomyces cerevisiae Rad54, a protein involved in recombinational repair and homologous recombination

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In the yeast *S. cerevisiae* repair of radiation induced DNA damage is under the control of several pathways. Among these the *Rad52* group genes (*RAD50-57, XRS2, MRE11*) are involved in recombinational repair. RAD54 gene leads to extreme X-ray sensitivity when mutated (Game & Mortimer [1974] Mutat. Res. 24, 281-292). With isogenic strains and *rad54Δ* mutations we demonstrated that Rad54 is required for mating type switching, a double-strand break mediated recombination event. Furthermore, *rad54Δ* cells exhibit a mutator effect and a reduced rate for spontaneous intragenic recombination at multiple genetic loci during mitosis.
The predicted Rad54 amino acid sequence (Emery et al. [1991] Gene 104, 103-106) suggested the existence of two potential nuclear localization signals, a potential leucine zipper, and a non-convential ATP binding site. Mutagenesis of this putative ATP binding site abrogates protein function in DNA repair and mating type switch demonstrating its functional significance. With anti-Rad54 polyclonal antibodies we can detect Rad54 protein in wild type cells in Western blots and using indirect immunofluorescence we have localized Rad54 protein in the nucleus. Over-expression of Rad54 protein in wild type cells causes a negative phenotype for MMS sensitivity, which suggest protein:protein interactions important for recombinational repair. We are currently purifying the native Rad54 protein from *S. cerevisiae* to determine its enzymatic activities.

S13-14

BINDING OF POLY(ADP-RIBOSE) TO PROTEINS: SEARCH FOR A SEQUENCE MOTIF

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Poly(ADP-ribose) is synthesized in mammalian cells following induction of DNA damage. This nucleic acid-like homopolymer may modulate chromatin functions by covalent and/or non-covalent interactions with nuclear proteins. The predominant protein species that bind poly(ADP-ribose) non-covalently are histones. Histone binding to poly(ADP-ribose) could be mediated by the histone tail domains.

Using a recently developed ADP-ribose polymer blot assay we tested several peptides for polymer binding. Analysing peptides with sequences similar to the core histone tail domains, we have found a positive signal for MARCKS psd peptide (25 aa), indicating that it binds poly(ADP-ribose) non-covalently. Further characterization of the polymer-binding motif was performed by altering the MARCKS psd peptide sequence.

S13-15

POLY(ADP-RIBOSE) METABOLISM AND RADIATION SENSITIVITY OF MOUSE LYMPHOMA CELL SUBLINES

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We analyzed the constitutive poly(ADP-ribose) metabolism in two closely related mouse lymphoma sublines L5178Y with differential radio-sensitivity: radiation resistant LY-R and radiation sensitive LY-S cells. Under no-stress conditions, the levels of NAD (the substrate for poly(ADP-ribose) polymerase) were identical in the two cell lines. LY-S cells contained more poly(ADP-ribose) polymerase protein (1.5 X) and more ADP-ribose polymers (2 X) than LY-R cells. This difference can be associated with the higher proliferative activity of LY-S cells. On the other hand, LY-S cells were missing at least one of the ADP-ribose binding proteins of about 50 kD. Absence of this protein could impair cellular response to DNA damage, contributing to the very high radiation sensitivity of LY-S cell line.

S13-16

MECHANISM OF RUVAB-PROMOTED DNA STRAND EXCHANGE.

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The RuvA and RuvB proteins of E. coli, which are induced in response to DNA damage, play important roles in the formation of heteroduplex DNA during genetic recombination and related recombinational repair processes. Together, RuvA and RuvB promote branch migration, an ATP-dependent reaction that increases the length of the heteroduplex DNA. Our electron microscopic studies showed that RuvB interacts with duplex DNA by forming hexameric rings encircling DNA molecules. We have visualized RuvAB complexes with Holliday junctions by electron microscopy and observed the formation of a tripartite protein complex in which RuvA binds the crossover and is sandwiched between two hexameric rings of RuvB. We propose a molecular model for branch migration, a novel feature of which is the role played by the two oppositely-oriented RuvB ring motors. The two ring motors, which are diametrically opposed across the Holliday junction, each encompass a DNA duplex and drive branch migration by catalyzing the directional unwinding and helical rotation of DNA. These studies provide new insight into the mechanism of DNA branch migration during genetic recombination and DNA repair.

S13-17

CHARACTERIZATION OF RECA-MEDIATED STRAND EXCHANGE REACTION BETWEEN TWO DUPLEX DNA MOLECULES.

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RecA protein *in vitro* can promote efficient reciprocal strand exchange between two duplex DNA molecules provided that one of the duplexes has a single stranded gap in the region homologous to terminal portions of the second duplex. The strand exchange apparently starts between double- and single-stranded region and later extends into duplex portions of the two molecules. In contrast to strand exchange between single- and double-stranded DNA which was successfully studied by electron microscopy, there are no such studies for duplex-duplex reaction. In particular it was not known if RecA covered portion on duplex DNA extends beyond the single-stranded gap and if yes, whether these portions of RecA-dsDNA complexes are able to bind and envelope a homologous duplex DNA.

Our studies with use of EM and nucleo-protein gels demonstrate that RecA filaments extends beyond the gap and that gapped duplex DNA is completely covered by RecA. EM studies of the reaction performed in the presence of slowly hydrolysable ATPγS indicate that only RecA complexes formed over a single-stranded region are able to specifically bind homologous duplex DNA. Further studies are in progress to elucidate the function of RecA filaments formed on double-stranded regions of gapped duplexes.

S13-18

Chromatin and Transcription Modulated Repair of UV Induced DNA Lesions in Yeast Minichromosomes

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To study the effect of chromatin structure on DNA repair, removal of UV lesions by nucleotide excision repair (NER) was analysed in a yeast minichromosome YRpTRURAP containing the URA3 gene and an ARS1 origin of replication. UV lesions were detected by primer extension with *Taq* DNA polymerase and quantified on sequencing gels using a PhosphorImager. The data show: (i) Fast repair on both strands in the nucleosome free promoter region. (ii) Fast and slow repair on the transcribed (TS) and non-transcribed (NTS) strand, respectively, consistent with transcription coupled repair. (iii) In the NTS, repair correlates with linker and nucleosomal regions, suggesting modulation by chromatin structure. (iv) Repair in the nucleosome free origin of replication (ARS1) was slow, suggesting inhibition of NER by bound factors.

S13-19

DEOXYRIBOSE MODIFICATIONS AS MOLECULAR TOOLS TO STUDY DNA DAMAGE-INDUCED MUTAGENESIS AND REPAIR

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Most physical or chemical mutagens react with DNA bases and disturb or disrupt their correct hydrogen bonding information. In many instances, these base lesions also distort the native conformation of the sugar-phosphate backbone of DNA. To examine the role of backbone conformation in determining the biological responses to base damage (mutagenesis, cytotoxicity, DNA repair), we constructed DNA molecules containing altered but stable deoxyribose residues (C4' modifications, L-deoxyribose, α-deoxyribose). In all cases, a single deoxyribose variant was introduced into DNA in a site-directed manner without affecting the chemistry of the corresponding base. We are currently using these site-specific deoxyribose substrates to investigate the recognition of DNA damage by DNA polymerases and repair enzymes at the molecular level. (Supported by the Wolfermann-Nägeli-Stiftung, Zürich)

S13-20

CHARACTERIZATION OF REC8, A MEIOTIC RECOMBINATION PROTEIN OF *S. POMBE*

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Rec8 is a gene with major functions in meiotic recombination since mutations in this gene reduce the frequency of meiotic intragenic recombination at the *ade6* locus 1000 fold to approximately the level of mitotic recombination. (De Veaux et al.,1992, Genetics **130** 251). Moreover, the *rec8* mutant shows precocious sister chromatid segregation at meiosis I and aberrant linear element formation (Molnar et al., Genetics **141** 61). The *rec8* gene has been cloned and sequenced, but the protein shows no sequence homology to any known proteins in the data-base (Lin et al., 1992, Genetics **132** 75).
We have obtained polyclonal antibodies by overexpressing the *rec8* gene in *E. coli* as a fusion protein and using this as antigen for immunization. Immuno-cytological experiments localize the protein to the nucleus of the cell. The protein is visible as foci in the nucleus.

Eukaryotic Transposable Elements (FEGS)

S14-01

CONTROL OF TRANSPOSITION IN DROSOPHILA

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Transposable elements make up a substantial proportion of most if not all eukaryotic genomes including man, usually comprising 10-15% of the total DNA. They occur as families of dispersed repeat sequences and can be classified according to their structure and presumed mechanism of transposition. There are two main classes, those that transpose by reverse transcription of an RNA intermediate and those that transpose directly from DNA to DNA. These elements normally move infrequently which is fortunate as they are potent mutagenic agents. In addition transposition of some elements is restricted to germ cells. This may also be advantageous for both the element and its host since transposition events in somatic cells might debilitate the individual in which they occur without increasing the number of elements transmitted to the next generation. The mechanisms that control transposition are complex and will be discussed with particular regard to elements in Drosophila that are responsible for hybrid dysgenesis.

S14-02

FONCTIONAL ANALYSIS OF A PLANT TRANSPOSABLE ELEMENT, THE Tnt1 RETROTRANSPOSON OF TOBACCO

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Retroviral-like elements are ubiquitous components of plant genomes. One of the few active autonomous plant retrotransposons is the tobacco Tnt1 element. Tnt1 expression is strongly induced by pathogen infections, and by biotic and abiotic elicitors, which all have in common to activate the plant defense response, and Tnt1 expression parallels the expression of early markers of the plant defense response. This regulation is maintained in heterologous plant species, and is mediated through regulatory sequences localized in the LTR U3 region.
The activation of transposable elements by genomic stresses has been documented in many animal or plant systems. Our results provide the first evidence of a direct influence of environnemental stresses such as pathogen attacks on the expression of a transposable element. The biological significance of Tnt1 specific regulation will be discussed.

S14-03

THE Tc1 TRANSPOSON OF THE NEMATODE *CAENORHABDITIS ELEGANS*

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The most widespread class of DNA transposons in the animal kingdom known today is that of the Tc1/*mariner* element. Sequence comparisons suggest that the element can hop from one species to the other, by horizontal transfer. We study the mechanism of Tc1 transposition, *in vivo* and *in vitro*. We recently developed a cell free system for Tc1 transposition. The reaction requirements are minimal: the other 26 base pairs of the transposon are sufficient for transposition. The reaction is carried out by transposase overproduced in nematodes, but also by transposase overproduced in insect cells. This shows that no other nematode-specific proteins than Tc1 transposase are required for transposition *in vitro*. This may explain the ease of horizontal transfer. It also suggests that Tc1 is a good tool for transgenesis of diverse animal species.

S14-04

HETEROLOGOUS EXPRESSION OF RETROTRANSPOSONS TO ANALYZE ELEMENT-ENCODED FUNCTIONS

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Retrotransposons multiply more often than the genome of their host by inserting copies of themselves into host DNA. The process involves reverse transcription of element RNA in cytoplasmic, virus-like particles and requires element-encoded functions, but also factors from the host.

In order to distinguish between element and host contributions, we expressed reading frames of the yeast element Ty1 in *E. coli*. Expression of a capsid-polyprotein fusion results in particles which contain reverse transcriptase and Ty1 RNA, supporting the notion that Ty1 particle formation and RNA packaging do not require host factors.