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 INA 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 INA. 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 Finnegan, D., Edinburgh

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_03

THE Tcl 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 nematodespecific 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.

S14-02

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.

S14-04

HETEROLOGOUS EXPRESSION OF RETROTRANSPOSONS TO ANALYZE ELEMENT-ENCODED FUNCTIONS

A. Bachmair, C. Luschnig, and S. Jelenic; Institut f. Botanik d. Univ. Wien, Rennweg 14, A-1030 Wien. 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 Tyl in *E. coli*. Expression of a capsid-polyprotein fusion results in particles which contain reverse transcriptase and Tyl RNA, supporting the notion that Tyl particle formation and RNA packaging do not require host factors.