Title

Loss of genomic integrity in human mammary epithelial cells

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SESSION VIII: DNA recombination, transposition, amplification

ORALS

O VIII.1 Protein-directed molecular interactions in recombination and repair

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Genetic recombination proceeds via a number of steps that involve defined protein-DNA interactions. In bacteria, many of the genes involved in recombination have been cloned and their protein products purified. The medial-to-late stages of recombination can be reconstituted in vitro using four purified proteins from *E. coli* (RecA, RuvA, RuvB and RuvC). In these reactions, RecA protein catalyses the pairing of two homologous DNA molecules and initiates strand exchange. Holiday junctions made by RecA are then recognised and processed by the Ruv proteins. RuvAB promote branch migration, dissociation of the RecA filament, and modulate the orientation of Holiday junction resolution by RuvC. Monoclonal antibodies raised against RuvA, RuvB or RuvC inhibit resolution in the reconstituted system indicating that the three Ruv proteins act as a complex. Additionally, specific protein-protein interactions between the branch migration motor (RuvB) and the resolvase (RuvC) have been observed. The structure and function of this complex in relation to its function will be discussed.

The biochemistry of homologous recombination in higher organisms is less well understood. However, the identification of proteins that are functionally homologous to their bacterial counterparts indicates that at least some recombination proteins have been conserved throughout evolution. A human gene that shares homology with *E. coli recA* (and its yeast homologue *RAD51*) has been cloned from a testis cDNA library, and its 37 kDa product (hRad51) purified to homogeneity. Human Rad51 binds to single- and double-stranded DNA and exhibits DNA-dependent ATPase activity. Complexes formed with single- and double-stranded DNA have been observed by electron microscopy following negative staining and exhibit the striated appearance characteristic of RecA or yeast Rad51 filaments. In vitro, hRad51 promotes ATP-dependent homologous pairing and strand exchange reactions. Strand exchange occurs with a 3′–5′ polarity, which is opposite to that promoted by RecA protein, and is stimulated by the presence of the single-strand DNA binding protein RP-A. These data indicate that hRad51 protein is likely to play an important role in the formation of recombinant DNA and/or the recombinational repair of damaged DNA in human cells.

O VIII.2 Meiotic recombination and human minisatellite rearrangements in the yeast *Saccharomyces cerevisiae*

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The maintenance of genomic stability requires the proper function of replication, repair, and recombination processes. Genetic recombination in meiosis contributes to enhance genetic diversity among progeny but also to ensure the faithful disjunction of the homologuous chromosomes at the first of the two meiotic divisions when the cells halve their chromosome content. In the past year, genetic and physical studies in the yeast *S. cerevisiae* have shown that meiotic recombination between homologs is initiated by the formation of transient DNA double-strand breaks (DSB), not only a few recombinational hot spots but all over the genome. The faithful repair of these numerous DSBs (at least one hundred per cell) is a genomic and cellular challenge for the meiotic cell to avoid the generation of inviable progenies.

Our recent results concern several aspects of the mechanism(s) and the control of genetic recombination and minisatellite rearrangements in yeast meiotics:

1. We have determined the distribution of meiotic DSBs along the 340 kilobases of chromosome III. We found 76 DSB regions mostly located in intergenic promoter-containing intervals. DSB regions are clustered in large (39-105 kb) chromosomal domains, both hot and cold (Baudat and Nicolas, 1997).
2. We found that the nuclease responsible for these meiotic DSBs is theSpo11 protein which shares sequence similarities to one subunit of a novel family of topoisomerase II discovered in the archaeon *Sulfolobus shibatae* (Bergerat et al., 1997).
3. In human, minisatellite sequnces are rearranged at high frequency in germ line.

To investigate the possible mechanism(s) of the instability of minisatellite repeats, we have integrated two human CEB1 alleles in the yeast genome. The features of their rearrangements in meiosis suggest that *S. cerevisiae* is a good model system.

Baudat, F. and Nicolas, A. (1997). Clustering of meiotic double-strand breaks on yeast chromosome III. Proc. Natl. Acad. Sci. (USA), 94, 5213–5218

Bergerat, A., et al., (1997), An atypical topoisomerase II from archaea with eucaryal homologues involved in meiotic recombination. Nature, 386, 414–417.

O VIII.3 Loss of genomic integrity in preneoplastic cells

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Genomic integrity is maintained by a network of cellular activities that assesses the status of the genome at a given point in time and provides signals to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. We have used viral proteins involved in malignant transformation as well as cells from patients predisposed to cancer, to investigate cellular pathways that may be perturbed during loss od genomic stability. Recent studies have identified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling noeplasia. Among the targets of the viral oncoproteins are the products of the p53 and retinoblastoma (Rb) tumor supressor genes. We previously demonstrated that the expression of human papillomavirus type 16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins, including Rb, resulted in a p53-independent alteration in cell cycle control, massive cell death and polyploidy upon PALA treatment. These results demonstrated that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells. More recent studies using cells from patients predisposed to cancer have focused on the enzymatic mechanisms that underlie the processes desribed above. The identification of these activities in human cells will be presented.