

Publication

A plasmid system to monitor gene conversion and reciprocal recombination in vitro

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A plasmid system allowing for the detection of recombinagenic activities in cell-free extracts is described. Two truncated alleles of the bacterial neomycin resistance gene (neo), differing from each other at a polymorphic restriction site, were constructed. Recombinations involving both alleles mediated by Drosophila embryo nuclear protein extracts or Drosophila larva whole cell protein extracts were selected by their ability to confer kanamycin resistance to E. coli. Restriction analysis of plasmids recovered from E. coli transformants allowed the monitoring of the two molecular mechanisms which can lead to functional neo genes, gene conversion and reciprocal recombination. A dose dependent increase in the recombination frequency with increasing amounts of cell extract was observed. Recombination was further increased by linearizing one of the two substrate plasmids. The Drosophila cell extracts catalyzed recombination in vitro since after incubation a recombination product could be identified by polymerase chain reaction (PCR) technology. The recombination was absolutely dependent on the presence of an active cell extract, since no diagnostic PCR product was detected in a reaction where extract was omitted. Analysis of a representative number of recombinant plasmids by restriction analysis revealed that in the absence of an exogenous recombinational system less than 2% of kanamycin resistant recombinant plasmids occurred by gene conversion upon transformation into E. coli. In contrast, recombinants exhibiting restriction patterns diagnostic for gene conversion were observed at frequencies between 5.1% and 9.8% after incubation with Drosophila larva cell extracts. These results strongly argued that gene conversion is a prominent mechanism of recombination in Drosophila mitotic cells.

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