End synapsis and replicative formation of IS*911* transposition intermediates.

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Transposition of the bacterial insertion sequence IS*911* involves the production of covalently closed circular intermediates which then undergo integration into a target DNA molecule. When the element is located on a plasmid a molecular species in the form of a figure-eight can be detected. This results from an initial single strand cleavage at one end (IR), liberating a 3'OH which acts as a nucleophile to attack the same strand three bases 5' to the other end.
We present the results of *in vivo* kinetic experiments which follow the formation and disappearance of this species. These demonstrate that the figure-eight is the precursor of the free IS circle. We have developed i*n vitro* systems which reproduce figure-eight formation and circle integration. We have been unable, however, to reproduce the formation of the circular IS from figure-eight intermediates *in vitro*. This suggested that specific host factors might be implicated in this reaction.
The *in vivo* kinetic data not only show that the figure-eight gives rise to a circular transposon form but also indicate that this step does not require IS*911* transposase. It must therefore involve host factors. We present experiments demonstrating that the primase, DnaG, is essential for this step, implicating replication in this conversion. Experiments using ^{3}H thymidine incorporation and plasmids with a temperature-sensitive replication apparatus demonstrated that the process does not require replication from the plasmid replication origin.
To investigate this potential replicative pathway for circle production more closely, we have used the *E.coli* replication terminator sequence, *terC*, which when bound by the Tus protein, inhibits replication forks in a polarised manner. When cloned within the transposon in an appropriate orientation, *terC* was found to inhibit figure-eight resolution and circle formation in the presence of Tus but not in its absence.
These results provide strong support for a model in which IS*911* circle intermediates are derived from the figure eight forms by a replicative mechanism. The results of studies using single molecule technologies to investigate synaptic complex formation between both transposon ends will also be presented.