Architecture of the Hin synaptic complex before and after DNA exchange

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The Hin recombinase catalyzes a site-specific DNA inversion reaction in the chromosome of *Salmonella* that regulates the alternative expression of two different flagellin genes. Although Hin is able to synapse the two *hix* recombination sites, the chemical steps leading to the exchange of DNA strands requires the formation of a higher order "invertasome" structure that contains the Fis-bound recombinational enhancer sequence. The invertasome is assembled at the base of a supercoiled DNA branch, which ensures that only *hix* sites on the same molecule recombine to invert the intervening DNA. We have isolated a series of hyperactive Hin mutants that no longer require Fis, the enhancer, or DNA supercoiling to promote recombination. These mutants have enabled development of a simplified in vitro system where reactions on oligonucleotide substrates can be studied. Synaptic complex intermediates can be isolated that contain 4 Hin protomers, each covalently associated with the 5' ends of the cleaved recombination sites through a phosphoserine linkage. These complexes can be chased into ligated products in which the DNAs are in either the parental or recombinant structures. Kinetic analysis reveals that synapsis, DNA exchange, ligation and resolution/disassembly are fast relative to the postsynaptic step(s) leading to DNA cleavage. Site-directed crosslinking between strategically-placed cysteines has been used to probe the configuration of Hin subunits within the synaptic complex during recombination. The results localize the interface between Hin dimers and demonstrate that the *hix* DNA segments are located on the outside of the complex. These experiments also provide evidence supporting a conformational change, such as a helix-coil transition, within the C-terminal end of the dimerization helices that may be critical for DNA attack by the active site serines. Crosslinked recombinants from differentially-tagged Hin homodimers demonstrate that heterodimers are formed upon DNA exchange. These results provides the first direct evidence for the exchange of protein subunits during recombination and are consistent with earlier experiments following topological changes in the DNA that occur upon recombination. The architecture of the Hin synaptic complex forces us to modify earlier structural models for the Fis-activated invertasome complex.