Studies on the Conjugative Transposon CTn DOT

Abigail A. Salyers, Nadja Shoemaker, Jeanne DiChiara, Yuri Sutanto, and Jeffrey F. Gardner * Department of Microbiology, University of Illinois, Urbana, IL 61801 USA

Conjugative transposons (CTns) are self-transmissible elements that are normally integrated into the bacterial chromosome. The first step in transfer is the excision of the CTn from the donor chromosome to form the circular transfer intermediate. A single strand of the intermediate is transferred to a recipient where it integrates into the recipients genome. The focus of our work is on a *Bacteroides* CTn, CTnDOT. CTnDOT is found in many human colonic *Bacteroides* strains. It carries genes that confer resistance to the antibiotics tetracycline and erythromycin. Previously we developed an *in vitro* system for the integration reaction. We also constructed a miniature (mini-element) form of CTnDOT that contains only the ends of CTnDOT and the CTnDOT integrase (IntDOT). Excision of the mini-element *in vivo* requires IntDOT and an operon that carries four genes whose gene products can function *in trans*. More recently we have developed an *in vitro* excision system. In this system, the left and right junctions of CTnDOT, *attL* and *attR*, are provided on separate plasmids. The excision reaction produces a cointegrate that contains the *attDOT* site (the joined ends of CTnDOT) and the chromosomal *attB* site. Optimal recombination frequencies were obtained from reactions that contained IntDOT and a crude extract from a *B. thetaiotaomicron* strain that carries the excision region of CTnDOT on a plasmid. Deletion analyses have determined the minimal DNA sequences of *attL* and *attR* that are necessary for recombination. Mutants that contain mutations in *attL* and *attR* that affect the efficiency of recombination have been isolated. The mutations may affect binding sites for IntDOT.