Analysis of Tn5 inversion events in *Escherichia coli* plasmids

Received: 30 October 1994 / Accepted: 26 January 1995

**Abstract** The ability of the bacterial transposon Tn5 to undergo sequence inversion in Rec⁺ *Escherichia coli* cells as a result of recombination between its duplicated IS50 elements was examined using specially designed plasmid constructs. Surprisingly, recombination events in the IS50 elements that led to crossover and therefore Tn5 inversion could be detected at a frequency of only 10⁻⁵. This was approximately an order of magnitude lower than the frequency of IS50 recombination that led to conversion events (i.e. non-reciprocal recombination) without crossover, and at least two orders of magnitude lower than the frequency of intermolecular recombination between IS50 elements on two different plasmids. These rare conversion and inversion events in Tn5 appeared to be due to intramolecular recombination and not simply to multiple rounds of reciprocal crossing over, since the heterodimeric intermediates that would be generated during the latter process could be readily isolated but were shown to yield a completely different set of plasmid products upon resolution.

**Key words** Conversion · IS50 · Recombination · Transposon

**Introduction**

Several well-studied pathways of homologous recombination exist in *Escherichia coli*. These include the RecBCD pathway, which is functional in wild-type *E. coli* strains and is inactivated by mutations in the *recBC* loci; the RecE pathway, which is activated in *recBC sbcA* mutant strains; and the RecF pathway, which is activated in *recBC sbcBC* mutant strains. Each of these pathways involves the contributions of the *recA* gene product and a host of other poorly characterized recombination enzymes, many of which appear to be utilized by more than one pathway (reviewed in Smith 1988, 1989). Plasmids have long been used as model substrates for studying the genetic and biochemical characteristics of these different routes of recombination. By manipulating regions of homology in plasmid constructs, the ability of the RecBCD, RecE, and RecF pathways to promote intermolecular recombination, which mediates plasmid oligomerization, and intramolecular recombination, which mediates inversion and deletion events, has been ascertained (Cohen and Laban 1983; Kolodner et al. 1985; Laban and Cohen 1981; Symington et al. 1985). This type of analysis has generally shown that while the RecBCD pathway contributes little to plasmidic recombination, both the RecE and RecF pathways appear to play significant roles in this process.

Since they typically contain duplicated insertion sequences, transposable elements should also be capable of undergoing homologous recombination events when present on bacterial plasmids. Tn5 is a well-characterized 5.8 kb transposon consisting of a 2.8 kb central unique region containing several antibiotic resistance genes, including a neomycin phosphotransferase (*neo*) gene, flanked by two inverted 1.5 kb IS50 insertion elements (Berg 1989). Inversion events which occur as a result of recombination between the duplicated IS50 elements of Tn5 have been examined in such diverse molecules as the *E. coli* chromosome (Berg 1980), the bacteriophage lambda genome (Yagil et al. 1980), the yeast 2 μm plasmid (Jayaram and Broach 1983), the herpes simplex virus type 1 genome (Weber et al. 1988a), and the SV40
minichromosome (Weber et al. 1990). In bacteria, this inversion is most probably mediated through one or more of the homologous recombination pathways of the host cell, since Tn5 lacks the site-specific resolvase function found in other transposons (Hirschel and Berg 1982).

In an earlier work (Weber et al. 1988b), the structure of Tn5 was manipulated to enable transposon inversion events to also be analyzed in *E. coli* plasmids. The present study utilized additional Tn5 constructions in an attempt to characterize the mechanism through which such plasmidic recombination occurs. It was determined from these analyses that the intramolecular recombination that mediates Tn5 inversion in plasmids is relatively rare when compared to either the intramolecular recombination which produces conversion events (i.e. non-reciprocal recombination) without sequence inversion, or the intermolecular recombination which is responsible for plasmid oligomerization. Moreover, the conversion and inversion events observed in this study did not appear to be the result of multiple rounds of reciprocal crossing-over between two plasmids, raising the possibility that true intramolecular recombination may be occurring in this plasmid in Rec+ *E. coli* cells.

### Results

Heterodimer formation, rather than true Tn5 inversion, is the preferred recombination event in cells carrying pTn5Δ1 (INV A).

Inversion of plasmid-borne Tn5 sequences was initially analyzed using the pTn5Δ1 (INV A) construct described in an earlier study (Weber et al. 1988b; Fig. 1A). The inversion of the 2.8 kb *BglII* fragment of Tn5 during the construction of this plasmid caused the neo gene of the transposon to fall under the control of the *neo* gene promoter of IS50<sub>LR</sub>, which is considerably weaker than its IS50<sub>L</sub> counterpart due to a single-base nonhomology between the two elements (Rothstein and Reznikoff 1981). However, inversion of Tn5 sequences due to intramolecular recombination between the IS50 elements of the plasmid should enable this weak *neo* gene to be expressed from the *lacZ* promoter in the pUC19 vector (Fig. 1A). This results in a significant increase in resistance to the antibiotic neomycin conferred on the host cell, as confirmed using a pTn5Δ1 (INV A) derivative in which Tn5 inversion was simulated by restriction fragment inversion (Weber et al. 1988b).

Two phenotypically distinct sizes of neomycin-resistant colonies were typically observed when the recombination-proficient *E. coli* strain C600 carrying pTn5Δ1 (INV A) was plated on YT media containing 35 μg/ml of neomycin. When plasmid DNA was isolated from smaller colonies and digested with *BamHI*, it appeared to lack both the predicted inversion event as well as any large-scale alternation of pTn5Δ1 (INV A) sequences; however, these isolates were always found to exist in a multimeric state when electrophoresed undigested. The apparent contribution of plasmid oligomerization to the activation of the *neo* gene in this isolates was characterized further in another study (Weber 1992). When plasmid DNA was purified from larger colonies and digested with *BamHI*, a set of fragments was invariably observed which appeared to be derived from an equal mixture of both the native and inverted forms of pTn5Δ1 (INV A); moreover, this DNA was found to exist exclusively in a dimeric state when electrophoresed undigested (Weber et al. 1988b, Fig. 1B). It was initially concluded that these plasmids represented homodimeric molecules in which one of the two copies of Tn5 had undergone sequence inversion (Weber et al. 1988b). However, further analysis of these plasmids using *EcoRI* (Fig. 1B) and other restriction endonucleases (unpublished results) revealed that they were actually heterodimeric molecules which were created by intermolecular recombination between the IS50<sub>L</sub> of one pTn5Δ1 (INV A) monomer and the IS50<sub>R</sub> of another (Fig. 1A). Although this recombination event did not represent the expected Tn5 inversion product, it nevertheless enabled one of the two *neo*