Increase in Conjugational Transmission Frequency of Nonconjugative Plasmids

Abstract. *Addition of Eco RI fragment 6 of the* Escherichia coli *sex factor F to pSC101 increases the frequency of its transmission by R1-19 and ColVB. Transmission frequencies of pSC101 and two pSC101 chimeras are also increased after the putative transposition of drug resistance element Tn3 from R1-19. These increases may result from addition of an origin of conjugational transfer to the plasmids.*

One problem in the containment of recombinant DNA is the possibility of transmission of chimeric plasmids to new bacterial strains by conjugation. Although the plasmid vectors used for cloning are not self-transmissible (that is, they are nonconjugative), these vectors can be transmitted to recipient strains during mating if a conjugative plasmid is also present in the donor cell. We refer to this process as mobilization of the nonconjugative plasmid by the conjugative one. The frequency of mobilization is different for each plasmid and varies over a wide range, from as low as 1×10^{-9} per donor for the vector pSC101 (*1*) to as high as 4×10^{-1} per donor for Col El (*2*). We were interested in the factors that determined the frequency of mobilization of nonconjugative plasmid vectors and chimeras, and whether a plasmid mobilized at low frequency, for example pSC101, could be converted to one mobilized at high frequency.

We have studied the mobilization of a series of chimeric pRS plasmids that were constructed in vitro from a partial Eco RI endonuclease digest of the conjugative plasmid F and a limit digest of the nonconjugative plasmid vector pSC101 (*3*) to see whether attachment of a fragment of F (the *Escherichia coli* sex factor) could allow pSC101 to be mobilized at high frequency. We expected that attachment of the transfer (*tra*) genes, required during mating for the production of pili and transfer-associated DNA metabolism (*4*), would be unlikely to affect the frequency of mobilization since all the *tra* functions would already be provided by any coresident conjugative plasmid. Previous findings from studies on F′ deletions indicated that when a large part or all of the region contained on Eco RI fragment 6 of F was deleted, the F′ became cis-dominant transfer deficient (*5, 6*), whereas a deletion or other mutation of any of the *tra* genes resulted in a recessive Tra$^-$ F′ (*5, 7*). Since fragment 6 appeared to affect the transfer ability of an F′, we examined the mobilization frequencies of pRS plasmids with and without fragment 6 of F.

Donor strains were constructed by transformation of a *recA*$^-$ F$^-$ strain of *E. coli* K12 with purified pRS plasmid DNA (*8*), followed by introduction of the mobilizing plasmid by conjugation. Table 1 shows the transmission frequencies for the pRS plasmids with ColVB or R1-19 (*9*) as conjugative mobilizing plasmids and with the *tet*$^+$ gene, which determines tetracycline resistance (Tc^R), as the selective marker. We used pSC105, a chimeric plasmid having a DNA fragment which confers resistance to kanamycin (Km^R) inserted into pSC101 at the Eco RI site rather than F fragments (*10*), as a control. Chloramphenicol resistance (Cm^R), determined by the *cat*$^+$ gene, was used to detect the transmission of R1-19. For the crosses involving R1-19 the addition of fragment 6 is sufficient for high frequency transmission of *tet*$^+$. With ColVB, fragment 6 increases the frequency 100- to 1000-fold, but fragment 3 appears also to be needed for maximum transmission of *tet*$^+$.

Previous results (*11*) indicated that low frequency mobilization of pSC101 occurred after a *recA*-independent recombination event between pSC101 and the conjugative plasmid pSC50 (*9*). A recent paper (*12*) demonstrated that this event is the transposition of the transposable element Tn3 from the plasmid pSC50 to pSC101. It is therefore possible that attachment of Eco RI fragment 6 to pSC101 increases the frequency of such a transposition and thereby increases the transmission frequency. On the other hand, it is possible that attachment of fragment 6 provides a site or gene required for a transposition-independent mobilization process, such as the initiation of rolling circle conjugational transfer replication (*4*). Since the evidence for transposition-mediated mobilization was the discovery that the *tet*$^+$ gene originally carried by pSC101 was found in transconjugant progeny on either of two new plasmids, each larger than pSC101 (*11*), we looked for such plasmids in the progeny from our crosses. The donors and Tc^R Cm^R progeny from each of the crosses involving R1-19 listed in Table 1 were screened for the presence of plasmid DNA. The donor strains showed two DNA bands which appeared to correspond to the separate nonconjugative and R1-19 plasmids as determined by R_F values (Table 2). The DNA patterns for the progeny, however, fell into two classes: (class I) progeny from donors

Table 1. Transmission frequencies of chimeric plasmids mobilized by ColVB or R1-19. *recA*$^-$ donor strains were grown in Luria (L) broth plus tetracycline (15 μg/ml) [plus chloramphenicol (25 μg/ml) for the R1-19 matings], washed one time with L broth, and mated in a 1 : 10 ratio with a *recA*56, F$^-$ strain (JC7199) for 1 hour at 37°C. Matings were interrupted by vortexing the mating mixture for 1 minute. Transmission of pRS and pSC plasmids was measured by selecting Tc^R (Pro$^+$ TL$^+$) progeny. Transmission of R1-19 was measured by Cm^R (Pro$^+$ TL$^+$) progeny. The numbers of Tc^R progeny and Cm^R progeny from the same donor were compared. ColVB transfer was not measured but >98 percent of the viable donor cells tested produced colicin and therefore carried the ColVB plasmid at the time of mating. Retransmission frequencies were measured as described above except that the recipient was the *recA*56F$^-$ strain, JC7214 and selection was for Tc^R (His$^+$ Met$^+$) or Cm^R (His$^+$ Met$^+$) progeny. Abbreviations: Tc, tetracycline; Cm, chloramphenicol; Pro, proline; TL, threonine + leucine; His, histidine; Met, methionine; R, resistant; $^+$, proficient; $^-$, deficient.

Chimeric plasmid	Eco RI fragments of F	Tc^R progeny per 100 donors mobilizing plasmid		Tc^R/Cm^R progeny	Retransmission frequency from progeny of R1-19 crosses	
		ColVB	R1-19		Tc^R progeny/100 donors	Tc^R/Cm^R progeny
pSC101		2×10^{-5}	2×10^{-2}	1×10^{-4}	5	4×10^{-1}
pSC105			3×10^{-3}	9×10^{-4}	3	4×10^{-1}
pRS5	7,5,3,6	2	6×10^{1}	5×10^{-1}		
pRS7	(17,19),2,12	8×10^{-5}				
pRS8	1(17,19),2,12,16,10	5×10^{-4}				
pRS21	5	5×10^{-5}	5×10^{-2}	5×10^{-4}	5×10^{-1}	4×10^{-1}
pRS27	6,15	2×10^{-2}	8×10^{1}	8×10^{-1}	2×10^{-1}	1
pRS30	3,6,15,1	7	6×10^{1}	6×10^{-1}		

that transmitted *tet*$^+$ at high frequency also appeared to show the original plasmid bands; (class II) progeny from donors which transmitted *tet*$^+$ at low frequency showed one band which appeared to correspond to R1-19 and a second band which had a lower mobility (and presumably larger size) than the original nonconjugative plasmid.

The presence of a presumably new plasmid band in progeny from donors carrying pSC101, pSC105, and pRS21, which are mobilized with low frequency, is in accord with the evidence of transposition-mediated mobilization obtained by Kopecko and Cohen (*11*). But since no new band was seen in the donor strains, we suspect that the transposition takes place in only a small number of the donor cells. High frequency mobilization, on the other hand, is characterized by the presence of plasmid bands with approximately the same mobility in both donor and recipient strains as would be expected if the nonconjugative plasmid were transmitted without having first to recombine with the conjugative plasmid. Therefore fragment 6 would appear to function not by increasing the frequency of transposition, but by providing something necessary for transmission which the mobilizing plasmid cannot provide.

To test further the idea that low frequency mobilization is preceded by a transposition event, progeny whose DNA had been analyzed on agarose gels were used as donors in matings to determine the frequency of retransmission of the *tet*$^+$ marker from these strains. If *tet*$^+$ had become associated with a high frequency transmissible plasmid, one would expect tetracycline resistance to be transmitted to a new recipient at high frequency. The results of these crosses are shown in the last two columns of Table 1. Transmission of *tet*$^+$ is 1000-fold higher for the strains carrying derivatives of the low frequency mobilized plasmids pSC101, pSC105, and pRS21 and nearly equals the transmission frequency of *cat*$^+$. Presumably these new plasmids are capable of being mobilized at high frequency. We have not yet determined which antibiotic resistance markers are carried by the new plasmids, but on the basis of the results of Kopecko *et al.* (*12*), we expect that they may show resistance to ampicillin which is carried on the Tn3 element. We are in the process of purifying plasmid DNA from all the progeny strains in order to compare the plasmids with their parents and to characterize them more fully. Our conclusions about the nature of these plasmids must be tentative until the more detailed results are in.

These data indicate that nonconjugative plasmid vectors and chimeras which are mobilized at low frequency can be converted to plasmids capable of high frequency mobilization. Such chimeric plasmids can be created in vitro by the addition to a plasmid vector of a DNA fragment providing a necessary protein or site. In the case of fragment 6 of F, we have hypothesized that the required site is the origin of transfer (*oriT*) of F since F′ plasmids with all or part of fragment 6 deleted have a cis-dominant transfer deficient phenotype (*13*). We have not ruled out the possibility that a gene on fragment 6 codes for a cis-acting protein. Plasmids which can be transmitted at high frequency can also be derived from the chimeric plasmid in vivo even in *recA*$^-$ strains (*11, 12*). The derivation could involve recombinational fusion of the two plasmids with subsequent recombinational separation in the donor or the progeny (*11*). Alternatively, there might be transposition of an *oriT* site from the conjugative to the nonconjugative plasmid in the donor. Since the recent findings of Kopecko *et al.* (*12*) show the transposition of the Tn3 from the conjugative plasmid pSC50 to pSC101, we think it is possible that Tn3, like fragment 6 of F, may carry an *oriT* which allows high frequency transmission of pSC101.

Note added in proof: We have purified plasmid DNA from the progeny of the R1-19 mobilizations of pSC101, pSC105, pRS21, and pRS27. The plasmids derived from mobilization of pSC101, pSC105, and pRS21 confer resistance to ampicillin and have an insertion in the pSC101 moiety of the plasmid as shown by Eco RI digestion and agarose gel electrophoresis. The plasmid derived from mobilization of pRS27 does not confer resistance to ampicillin and has the original plasmid bands with no DNA insertion.

Table 2. Results of agarose gel electrophoresis of DNA prepared from donor and progeny strains from crosses in which R1-19 was the conjugative plasmid. The DNA was prepared by the method of Meyers *et al.* (*14*) from 30-ml cultures grown in Luria broth containing tetracycline (15 μg/ml) and chloramphenicol (25 μg/ml), and was analyzed on 0.7 percent agarose gels. Linear pVH51 DNA was added to each well as an internal standard for R_F determinations. The R_F values for each plasmid band were calculated as the ratio of the distance from well to the distance from well of pVH51. When the R_F of the band assumed to be R1-19 differed from well to well, the R_F of the second band differed correspondingly in class I results. DNA preparations were also made from strains carrying each plasmid separately, and the following R_F values for each plasmid were obtained: R1-19, 0.29; pRS5, 0.39; pRS27, 0.51; pRS30, 0.40; pSC101, 0.70; pSC105, 0.52; pRS21, 0.50. Several slab gels were run to obtain these results and those shown in the table.

Class	Conjugative plasmid	Non-conjugative plasmid	R_F of donor plasmids	R_F of progeny plasmids	Tentative conclusion
I	R1-19	pRS5	0.28	0.31	Both R1-19
			0.36	0.38	Both pRS5
I	R1-19	pRS27	0.29	0.29	Both R1-19
			0.46	0.45	Both pRS27
I	R1-19	pRS30	0.26	0.31	Both R1-19
			0.33	0.37	Both pRS30
II	R1-19	pSC101	0.31	0.31	Both R1-19
			0.66	0.50	New plasmid in progeny
II	R1-19	pSC105	0.30	0.30	Both R1-19
			0.52	0.46	New plasmid in progeny
II	R1-19	pRS21	0.30	0.30	Both R1-19
			0.54	0.42	New plasmid in progeny

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9. The R1-19 plasmid used here was obtained from D. R. Helinski and does not express resistance to kanamycin. It appears to be similar to the pSC50 plasmid isolated by Kopecko and Cohen (*11*). An R1-19 plasmid obtained from N. S. Willetts expresses resistance to kanamycin and mobilizes all the chimeric plasmids tested at low frequency (10^{-2} to 10^{-4} per 100 donors). The reason for the difference between the mobilization properties of these two R1-19 plasmids has not been determined.
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