JOURNAL OF BACTERIOLOGY, July 1994, p. 4455–4458
0021-9193/94/$04.00+0

Vol. 176, No. 14

Requirements for Mobilization of Plasmids RSF1010 and ColE1 by the IncW Plasmid R388: *trwB* and RP4 *traG* Are Interchangeable

ELENA CABEZÓN,[1] ERICH LANKA,[2] AND FERNANDO DE LA CRUZ[1]*

Departamento de Biología Molecular, Universidad de Cantabria, 39011 Santander, Spain,[1] and Max-Planck-Institut für Molekulare Genetik, D-14195 Berlin, Germany[2]

Received 13 January 1994/Accepted 11 May 1994

Mobilization of plasmid RSF1010 by the IncW plasmid R388 requires the genes involved in W pilus synthesis plus *trwB*. *traG* of the IncP plasmid RP4 can substitute for *trwB* in RSF1010 mobilization by R388 but not in self-transfer of R388. This result suggests a dual specificity of TrwB-like proteins in conjugation. The same genetic requirements were found for R388 to mobilize the unrelated plasmid ColE1.

Bacterial conjugation is the process whereby DNA is transferred from one bacterial cell to another by a mechanism which requires physical contact between the mating cells (5). The genetic information required for this process is encoded by plasmids in gram-negative bacteria. Besides self-transfer, conjugative plasmids can promote the transfer of nonconjugative plasmids by mobilization. At least 20 different groups of naturally occurring self-transmissible plasmids occur in members of the family *Enterobacteriaceae*, and a large number of mobilization systems may exist. Mobilizable plasmids are best exemplified by plasmids RSF1010 (14) and ColE1 (4). To be mobilized by a self-transmissible plasmid, both RSF1010 and ColE1 contain a specific set of functions that enable mobilization, i.e., an origin of transfer (*oriT*) and three genes encoding the so-called mobilization proteins (Mob proteins) (3, 13). It is assumed that the self-transmissible plasmid provides the pilus machinery for the cell-to-cell interactions and DNA transport. The Mob proteins are required for the specific recognition and processing of *oriT*. However, very few data are available about what *tra* genes of the transmissible plasmid are required, and which are dispensable, for mobilization. For instance, the Tra2 genes and the *traG* and *traF* genes of Tra1 are sufficient for RSF1010 mobilization by RP4 (9, 10). In an analogous way, Ti plasmid-encoded VirD4 together with the VirB functions, normally involved in T-DNA transfer to plant cells, can direct conjugative transfer of RSF1010 between agrobacteria (1). Furthermore, mobilization of ColE1 by plasmid F requires the genes involved in pilus formation plus *traD*, while *traI*, *traM*, and *traZ* are not required (19). An interesting problem arose when it was reported that plasmid RSF1010 could be mobilized by the IncP plasmid RP4 at high frequency but very poorly by the IncW plasmid R388 (20). On the other hand, ColE1 was mobilized by R388 better than it was by RP4 (15). These results suggest that there are specific interactions between the RSF1010 and the ColE1 relaxosomes and the different transfer apparatuses. A better knowledge of these interactions will help us understand both the molecular mechanism of conjugative DNA transfer and the reasons for the ecological prevalence of different self-transferable and mobilizable plasmids. The experiments reported here were designed to unravel the differential interactions of the IncP and IncW transfer systems for the mobilization of RSF1010 and ColE1.

R388 genes required for RSF1010 mobilization. RSF1010K is a derivative of RSF1010 by insertion of a fragment that encodes a Km^r gene in place of the Sm^r gene (9). Mobilization experiments were carried out in derivatives of *Escherichia coli* DH5α containing RSF1010K and different conjugative plasmids. They were mated with the recipient strain UB1637 for 1 h on solid medium as described previously (2). Transconjugants were selected on streptomycin (to counterselect donors) plus the antibiotic resistance marker of the individual plasmids to be tested. Table 1 shows the results of the RSF1010 mobilization experiments. RP4 is efficient in RSF1010 mobilization, while R388 is about 300 times less efficient. The products of R388 mobilization are, however, bona fide mobilization products, that is, they contain both plasmids of the donor strain without any modification in their physical structure (data not shown).

We next wanted to find out which R388 genes were required for RSF1010K mobilization. Figure 1 shows the genetic organization of the R388 transfer system and of the R388-derived plasmids used in this study. pSU4053, which contains all of the TRA_w region, mobilized RSF1010K at the same frequency as the wild-type R388. However, pSU1424, which contains only PIL_w, is incapable of mobilizing RSF1010. To find out what other genes, apart from those in PIL_w, are required for mobilization, transfer of RSF1010K by R388 mutants in each of the three genes in MOB_w was checked. Table 1 shows that, while *trwB* mutants are completely Mob^-, *trwA* and *trwC* are dispensable. The latter result also demonstrates that mobilization and self-transfer are uncoupled. The same mobilization frequencies are obtained with different *trwC* mutants irrespective of their frequencies of self-transfer.

TraG protein of RP4 can substitute for TrwB of R388 in RSF1010 mobilization. Mobilization of RSF1010 by plasmid RP4 requires the Tra2 genes involved in pilus assembly and two Tra1 genes, *traF* and *traG*. TraF, but not TraG, is required for P pilus assembly (17). *traF* and *traG* are contained in the recombinant plasmid pBS103 (21). Table 1 shows that pBS103 can complement mobilization of RSF1010 by pSU1424 to wild-type frequencies. The RP4 genes *traF* and *traG* were cloned separately, giving pWP471 and pBS140 (17). pBS140 ($traG^+$) complements pSU1424 as efficiently as does pBS103, while pWP471 ($traF^+$) does not have any effect. From this result, it can be concluded that R388 TrwB and RP4 TraG

* Corresponding author. Mailing address: Departamento de Biología Molecular, Universidad de Cantabria, C. Herrera Oria s/n, 39011 Santander, Spain. Phone: 34-42-201942. Fax: 34-42-201945. Electronic mail address: delacruz @ ccucvx.unican.es.

TABLE 1. Mobilization of plasmid RSF1010K by different plasmids[a]

Plasmid(s)	Transconjugants/ml		Mobilization frequency[b]
	Conjugative plasmid	RSF1010K	
RP4	2.3×10^8	1.6×10^8	7.0×10^{-1}
R388	5.7×10^6	9.3×10^3	1.6×10^{-3}
R388, pBS103	8.1×10^6	2.4×10^4	2.9×10^{-3}
pSU4053	8.5×10^6	4.5×10^4	5.3×10^{-3}
pSU4028	1.0×10^2	3.0×10^4	3.0×10^2
pSU1456	<10	<10	
pSU1457	<10	<10	
pSU1458	3.4×10^1	1.6×10^4	4.7×10^2
pSU1459	1.7×10^4	1.6×10^4	9.4×10^{-1}
pSU1511	5.1×10^6	1.0×10^4	2.0×10^{-3}
pSU1424		<10	
pSU1424, pBS103		3.8×10^4	
pSU1424, pBS140		9.8×10^4	
pSU1424, pWP471		<10	
pSU1424, pSU1482		5.7×10^3	
pSU1456,[c] pBS103	3.4×10^1		
pSU1457,[c] pBS103	5.0×10^1		

[a] Derivatives of DH5α (7) carrying RSF1010K and the plasmids shown in the first column were mated with UB1637 for 1 h at 37°C on solid medium. The cells were resuspended, diluted, and plated on selective plates as described previously (2).

[b] The mobilization frequency is the number of Km^r transconjugants (containing RSF1010K) divided by the number of transconjugants containing the reporter gene of the conjugative plasmid. The values represent the average of at least three experiments.

[c] In these two cases, the donor strains contain only pBS103 and the *trwB* mutant of R388.

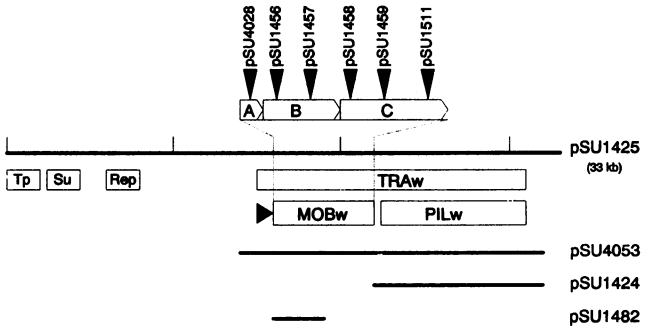

FIG. 1. Physical and genetic structure of various plasmids derived from R388. pSU1425 is R388 lacking the single *Eco*RI site (12). pSU4053 contains all of the genes required for R388 conjugative DNA transfer (TRA_w region) cloned in the intermediate-copy-number vector pHG327 (2). pSU1424 contains the genes required for pilus synthesis and assembly (PIL_w region) subcloned in the vector pSU19 (2). The MOB_w region is composed of three genes (*trwA*, *trwB*, and *trwC*) and the origin of transfer (*oriT*). pSU1482 contains the *trwA* and *trwB* genes subcloned in the expression vector pKK223-3 (11). Horizontal lines represent the extent of pSU1425 contained in these recombinant plasmids. Plasmids pSU1456 to pSU1511 contain insertions of 72 bp in pSU1425 so that they produce in-frame insertions of 24 amino acids in the respective protein (TrwB or TrwC). pSU1456, pSU1457, and pSU1458 are Tra$^-$. pSU1459 shows a frequency of transfer of 3×10^{-3} relative to pSU1425, and pSU1511 is Tra$^+$. pSU4028 contains a 68-bp insertion in *trwA* (2), which results in a truncated TrwA protein, and shows a transfer frequency of 2×10^{-5} relative to pSU1425.

perform equivalent roles in mobilization. It can also be inferred from this result that *traG* would complement R388 *trwB* mutants for self-transfer. However, transfer of the R388 *trwB* mutants pSU1456 and pSU1457 by pBS103 was 10^5-fold lower than the wild-type conjugation frequency (Table 1). Plasmid DNA obtained from transconjugant colonies contained intact pSU1456 or pSU1457, suggesting that some complementation did in fact occur, although at low efficiency. Considering the relatively low mobilization frequencies of RSF1010 by R388 or by the *vir* genes of the Ti plasmids (1) in relation to RP4 (Table 1), it was suggested that protein TraF of plasmid RP4 could be responsible for the enhanced efficiency of mobilization (9). However, our results show that pBS103 did not enhance R388 transfer. Furthermore, the same mobilization frequencies are obtained with pBS140 as with pBS103 in the complementation of RSF1010K mobilization by pSU1424. Therefore, if TraF is important for the efficiency of RSF1010 mobilization by RP4, it cannot act in the IncW system.

Mobilization of the unrelated plasmid ColE1 by R388 requires the same set of genes. The ColE1 Mob proteins are not related to the RSF1010 Mob proteins (4, 6). Therefore, it seemed adequate to analyze whether R388 could interact with ColE1 in the same or a different manner than it could with RSF1010. Plasmid pSU4601 contains a 2-kb fragment carrying the Km^r gene from pUC4K inserted in the *Eco*RI site of ColE1. The mobilization of pSU4601 by different derivatives of R388 was assayed as described for RSF1010K, and the results are shown in Table 2. It is shown that *trwA* and *trwC* are also dispensable for ColE1 mobilization, while *trwB* and PIL_w are again required. ColE1 is mobilized by R388 at about the same frequency as RSF1010 is. This result contrasts with the efficiency of RP4 mobilization. In this case, mobilization of ColE1 is less efficient (15) than RSF1010 mobilization.

The role of TrwB-like proteins in conjugal DNA transfer. The results presented above show that mobilization of both ColE1 and RSF1010 by R388 requires the genes for pilus formation (PIL_w genes) plus *trwB*. Similarly, mobilization of RSF1010 by RP4 requires the Tra2 genes plus *traF* and *traG* (9). The Tra2 genes and *traF* are required for RP4 pilus formation; therefore, presumably, *traG* performs a role equivalent to that of *trwB*. This presumption has been confirmed by the complementation of *trwB* mutants by TraG (Table 1). Besides, it has also been reported that mobilization of ColE1 by plasmid F requires all of the *tra* genes involved in pilus formation plus *traD*. Strikingly, mobilization of the ColE1-related plasmid CloDF13 was independent of TraD (19). TrwB, together with TraG of RP4, TraD of plasmid F, and VirD4 of the Ti plasmid, form a family of proteins essential for

TABLE 2. Mobilization of nonconjugative plasmid pSU4601 (ColE1 Km^r) by R388 and derivatives[a]

Plasmid(s)	Transconjugants/ml		Mobilization frequency
	Conjugative plasmid	pSU4601	
R388	8.1×10^6	1.2×10^4	1.5×10^{-3}
pSU4028	2.3×10^1	1.6×10^4	7.0×10^2
pSU1456	<10	<10	
pSU1457	<10	<10	
pSU1458	1.5×10^2	3.5×10^4	2.3×10^2
pSU1459	4.5×10^4	5.4×10^4	0.12×10^1
pSU1511	1.0×10^7	4.6×10^4	4.6×10^{-3}
pSU1424		<10	
pSU1424, pSU1482		5.7×10^3	

[a] Experiments were carried out and frequencies were calculated as described in Table 1, footnotes *a* and *b*.

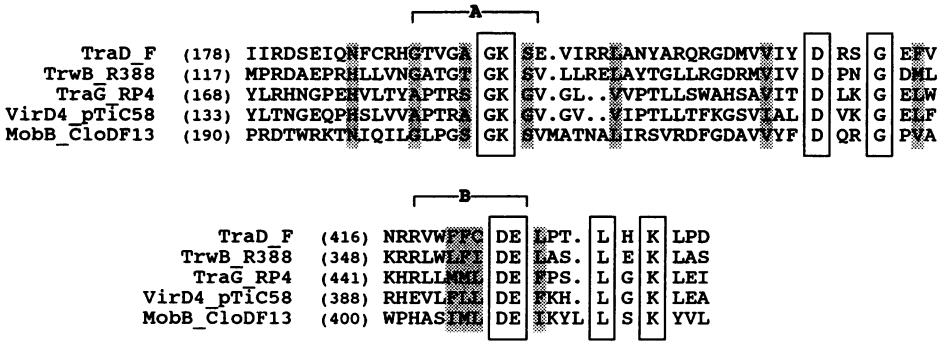

FIG. 2. Conserved amino acid motifs in the TrwB family of conjugation proteins. The five proteins shown share the nucleoside triphosphate-binding domains A and B described previously (12, 16). Invariant amino acids are boxed. Related amino acids are shaded. Data library accession numbers: TraD of plasmid F, PO9130; TrwB of plasmid R388, translated from EMBL X63150; TraG of plasmid RP4, translated from EMBL X54459; VirD4 of plasmid pTiC58, P18594; MobB of plasmid CloDF13, PO8098.

conjugal DNA transfer which show conservation of the primary amino acid sequence (12). Therefore, we wondered if CloDF13 could have a protein that acted as a TraD analog. Figure 2 shows that this is probably the case since the protein MobB of CloDF13 (which has no counterpart in ColE1 or RSF1010) shows conservation of two domains present in the TrwB-like proteins and possibly implicated in nucleotide binding (16). However, it should be pointed out that analysis of the amino acid sequence of MobB predicts it to be a soluble cytoplasmic protein, while the rest of the members of the family are integral membrane proteins.

Here we show that TraG effectively complements *trwB* mutants for RSF1010 mobilization but not for R388 self-transfer. This result is interesting since it suggests a dual specificity for TrwB in conjugation. There is a process, in which TrwB and TraG are interchangeable, that is related to the mobilization of RSF1010, and there is a second one in which TraG cannot substitute for TrwB in recognition or processing of the R388 relaxosome. The fact that pBS103 does not interfere with R388 $trwB^+$ self-transfer (Table 1) suggests that TraG fails to interact with R388 self-transfer functions (a nonproductive interaction, on the contrary, would result in a dominant negative "mutant" of *trwB*). It may be speculated that a Mob protein of RSF1010 performs this specificity function (presumably related to *oriT* recognition or processing) and that the relaxosome can be further processed by a variety of TrwB-like proteins.

Some speculation is also warranted by the fact that RSF1010 and ColE1 mobilization is independent from the conjugative helicases (as shown here for TrwC of R388 and in reference 19 for TraI of plasmid F). Furthermore, an RP4-encoded helicase has not been identified (8). However, present-day models for the mechanism of conjugal transfer of DNA all involve a single-stranded DNA intermediate which is displaced from the donor DNA molecule by rolling-circle-type replication (18). Where does the helicase that is undoubtedly required for processing of the mobilizable plasmids come from? Two alternatives seem plausible: either plasmid mobilization involves a host-encoded helicase or the TrwB-like proteins participate, directly or indirectly, in the unwinding reaction.

We expect that further dissection of the interactions involved in plasmid mobilization will shed more light on the molecular mechanism of bacterial conjugation.

This work was supported by a grant (BIO93-0371) from the C.I.C.Y.T. (Madrid, Spain) to F.C. E.C. is a recipient of a predoctoral fellowship from the Government of the Basque Country. E.L. is indebted to Heinz Schuster for generous financial support.

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