Identification and Properties of a Novel *clt* Locus in the *Streptomyces phaeochromogenes* Plasmid pJV1

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A novel *clt* locus required for efficient transfer of the *Streptomyces phaeochromogenes* plasmid pJV1 was identified and mapped. The *clt* region was functional in both orientations, and its absence caused a severe reduction in plasmid transfer. Chromosome mobilization, on the other hand, was not affected by absence of the *clt* locus. The *clt* region showed structural, but not sequence, similarity to transfer origins of gram-negative plasmids.

Most *Streptomyces* plasmids are self-transmissible and endowed with chromosome mobilizing ability (CMA), which accounts perhaps for all fertility observed in this genus (7). Knowledge about these events at the molecular level is still scarce, even though transfer and CMA require only a few plasmid-borne genes, at least in the case of the small circular *Streptomyces* plasmids (6). It is not known whether transfer of *Streptomyces* plasmids resembles conjugative transfer in gram-negative bacteria, in which only one plasmid strand is transferred to the recipient cell during an event of rolling-circle replication. Such an event is initiated when specific transfer proteins create a single-stranded nick inside the origin of transfer (*oriT*) (19). A *cis* locus for transfer (*clt*) that could be a functional analog of an *oriT* has been described for pIJ101; this locus, however, shows no structural or sequence similarity to *oriTs* of gram-negative bacterial plasmids (14).

The 11-kb circular plasmid pJV1, isolated from *Streptomyces phaeochromogenes* (1), replicates via single-stranded intermediates by a rolling-circle mechanism (15, 16). Intermycelial plasmid transfer requires the products of the pJV1 *traA* and *traB* genes, whose expression is regulated by the product of *traR*; in addition, the products of the pJV1 *spdB* genes are required for colonization of the recipient mycelium, leading to a "pock" formation (16). Here I describe another pJV1 locus, *clt*, which is also necessary for efficient plasmid transfer. The pJV1 *clt* locus resembles in function, but not in sequence, the *clt* locus of pIJ101 and shows some structural similarity to an *oriT*.

Localization of a pJV1 locus essential for efficient plasmid transfer. A previous functional analysis of pJV1 suggested that efficient plasmid transfer required, apart from functional *tra* genes, a region located in the vicinity of the *spdB* genes; its absence caused a 10^4 -fold reduction in transfer frequencies compared with plasmids with deletion and frameshift mutations affecting only the *spdB* genes (16). To confirm the presence of this locus and to further localize it, a series of plasmid derivatives were constructed (Table 1 and Fig. 1); these derivatives were introduced into *Streptomyces lividans* TK64, and their transfer and CMA frequencies were analyzed in crosses with *S. lividans* TK54, as previously described (16). From these

results (Table 2) it is clear that the region required for efficient transfer is contained in the 200-bp *EcoRI*-*SstI* fragment (sites 1 to 2 in Fig. 1), which consists mostly of the *traB*-*spdB1* intercistronic region (16). In order to test whether this was the only region (apart from the *tra* genes) required for efficient plasmid transfer, its effect was analyzed in a minimal replicon derivative by providing the pJV1 transfer functions in *trans* from a chromosomal location. To do this, the pJV1 transfer genes were cloned into the lysogenic ϕ C31 derivative KC304 (5) as described in Table 1. The phage thus constructed, KCBM1 (Fig. 1), was then used to obtain thiostrepton-resistant lysogens of *S. lividans* TK64; a similar phage, KCBM2, that carried an internal deletion affecting all *tra* genes (Table 1 and Fig. 1) was used to obtain a control TK64 lysogen. In order to prevent zygotic induction of KCBM1 in crosses, the recipient strain TK54 was also lysogenized with KCBM2 (no stable KC304 lysogens could be obtained in either TK64 or TK54, which could be related to the presence of *vph* sequences and/or to the small size of the phage [2]). TK64(KCBM1) lysogens showed CMA which was only slightly lower than that obtained when the transfer functions were expressed from the plasmid, whereas TK64(KCBM2) lysogens were infertile (Table 2), indicating that all fertility in crosses involving KCBM1 lysogens resulted from the cloned pJV1 transfer functions. Plasmid pB48, a minimal replicon derivative from pJV1 (Table 1 and Fig. 1), was introduced into TK64(KCBM1) lysogens and crossed with TK54(KCBM2); plasmid transfer occurred with a low frequency similar to that observed for pB69 and pB80 (Table 2). When the 200-bp *EcoRI*-*SstI* fragment was subcloned into pB48 and the resulting plasmid, pB91, was introduced into TK64(KCBM1), the transfer frequency increased over 10^4 -fold (Table 2), confirming that this was the only plasmid region required for efficient transfer; this experiment also showed that its function is independent of orientation, since in pB91 this fragment has an orientation opposite that of the wild type. This region, therefore, is best described as the pJV1 *cis* locus for transfer (*clt*) since it resembles in function the *clt* locus of pIJ101 (14).

Analysis of the *traB*-*spdB1* intercistronic region. Analysis of the pJV1 *clt* region failed to reveal significant similarity either to pIJ101 *clt* or to consensus sequences surrounding the *nic* sites of conjugative plasmids from different sources (3, 10, 13, 17). Database searches (data not shown) revealed significant similarity of the pJV1 *clt* sequence only to the equivalent region of the *Streptomyces nigrificans* plasmid pSN22 (11), which

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TABLE 1. Bacterial strains, plasmids, and bacteriophage used in this study

Strain, plasmid, or phage	Description	Reference
<i>S. lividans</i>		
TK64	<i>pro-2 str-6</i> SLP2 ⁻ SLP3 ⁻	8
TK54	<i>his-2 leu-2 spc-1</i> SLP2 ⁻ SLP2 ⁻ SLP3 ⁻	8
Plasmids		
pB48	Derivative of the Hyg ^r minimal pJV1 replicon pB45 (16) with the 1.5-kb <i>Bcl</i> I fragment from pIJ702 carrying the <i>melC</i> genes (12) inserted into the <i>Bgl</i> II site	This work
pB50	Insertion of a 1.1-kb <i>Bcl</i> I fragment carrying the thiostrepton resistance gene (<i>tsr</i>) from pIJ486 (18) into the single <i>Bgl</i> II site of wild-type pJV1	16
pB69	pB50 with the <i>Eco</i> RI- <i>Eco</i> RV fragment comprising the <i>spdB</i> region and part of the <i>tsr</i> gene replaced by the <i>Eco</i> RI- <i>Eco</i> RV fragment from pIJ486 (18)	16
pB78	Deletion of the <i>Kpn</i> I- <i>Not</i> I fragment of pB50 (sites 3 to 4 in Fig. 1) affecting the three <i>spdB</i> genes but not their flanking regions	This work
pB79	Deletion of the <i>Not</i> I- <i>Bam</i> HI fragment of pB50 (sites 4 to 5 in Fig. 1) affecting <i>spdB3</i> and the downstream flanking region	This work
pB80	Deletion of the <i>Eco</i> RI- <i>Kpn</i> I fragment of pB50 (sites 1 to 3 in Fig. 1) affecting <i>spdB1</i> and the upstream flanking region	This work
pB81	200-bp <i>Eco</i> RI- <i>Sst</i> I fragment (sites 1 to 2 in Fig. 1) inserted into pB69 as an <i>Eco</i> RI- <i>Pst</i> I fragment, after passage through the pIJ2925 polylinker (9)	This work
pB91	200-bp <i>Eco</i> RI- <i>Sst</i> I fragment (sites 1 and 2 in Fig. 1) inserted into pB48 as a <i>Bgl</i> II- <i>Pst</i> I fragment, after passage through the pIJ2925 polylinker (9)	This work
Phage		
KCBM1	3.9-kb <i>Mlu</i> I- <i>Eco</i> RI fragment (sites 9 to 1 in Fig. 1) comprising the pJV1 <i>tra</i> genes cloned as a <i>Bgl</i> II fragment into the <i>Bam</i> HI site of KC304 (5), after passage though the pB72 polylinker (16); carries a <i>tsr</i> gene for lysogen selection	This work
KCBM2	Same as KCBM1 but carrying an internal <i>Bpu</i> 1102I- <i>Bam</i> HI deletion (sites 10 and 11 in Fig. 1) which affects the three <i>tra</i> genes	This work

FIG. 1. Linear representation of pJV1 and derivatives. The plasmid is represented linearized at the single *Eco*RI site. See reference 16 for a description of the different plasmid genes and loci. Portions of the different derivatives not originating from pJV1 are shown as black lines. Arrows point in the direction of transcription of the different genes, except for phage derivatives, where they show the orientation of the vector relative to the insert. Note that the *Eco*RI-*Sst*I fragment in pB91 is inverted. Not all *Mlu*I, *Not*I, and *Sst*I sites are shown.

FIG. 2. Nucleotide sequence of the pJV1 *traB-spdB1* intercistronic region. The sequence shown corresponds to nucleotides 11075 to 201 of the pJV1 sequence (16), which has the GenBank accession number U23762. Direct repeats are shown by thin arrows above the sequence, whereas the imperfect inverted repeats are shown by thick arrows below the sequence. The ribosome-binding site of *spdB1* is underlined, as are the *EcoRI* and *SstI* sites, which correspond to sites 1 and 2 in Fig. 1, respectively.

was expected, given the high level of homology between their transfer and spread genes (16). Although no significant sequence similarities with described *oriT* sequences were observed, the pJV1 *clt* region does contain a series of direct repeats (with the sequence CCGCACA[C/G][C/G]), as well as two pairs of imperfect inverted repeats (Fig. 2); this resembles the situation in *oriT* regions, where repeats of this type are commonly observed (19) and where some have been shown to function as target sequences for protein binding (4, 20). Therefore, there is at least some structural similarity between pJV1 *clt* and *oriT* regions. It is worth mentioning that the four direct repeats located to the left of the *EcoRI* site may not be necessary for pJV1 *clt* function, as indicated by the efficient transfer of pB91. The presence of direct and inverted repeats in pJV1 *clt* is in contrast to the situation in pIJ101 *clt*, in which no significant sequences of this type were observed (14).

The pJV1 *clt* locus is not required for CMA. In pIJ101 the absence of *clt* reduced CMA by several orders of magnitude when the transfer functions were expressed from the plasmid, which was attributed to an effect in *cis* on *tra* gene expression (14). In contrast, when the pJV1 transfer functions were expressed from either the plasmid or the chromosome, CMA was not significantly affected by the absence of the cognate *clt* locus (Table 2); in both cases, the absence of *clt* resulted in only a small decrease in fertility. Therefore, it is unlikely that the presence of pJV1 *clt* in the plasmid has any effect in *cis* on expression of the transfer genes. The fact that CMA frequencies were similar irrespective of the plasmid transfer frequency, coupled with the similar CMA frequencies found when the transfer genes were expressed from a chromosomal location (Table 2), argues against conduction playing a significant role in chromosome mobilization by pJV1.

TABLE 2. Transfer and CMA properties of pJV1 derivatives in *S. lividans*

Donor	Plasmid	Recipient	Transfer frequency ^a	CMA ^b
TK64	pB50	TK54	9.3×10^{-1}	6.5×10^{-6}
TK64	pB69	TK54	5.6×10^{-5c}	1.6×10^{-6}
TK64	pB78	TK54	1.4×10^{-1}	3.2×10^{-6}
TK64	pB79	TK54	2.5×10^{-1}	5.9×10^{-6}
TK64	pB80	TK54	1.5×10^{-5c}	7.7×10^{-7}
TK64	pB81	TK54	2.6×10^{-1}	3.6×10^{-6}
TK64(KCBM2)	None	TK54(KCBM2)	NA ^d	$<10^{-8}$
TK64(KCBM1)	None	TK54(KCBM2)	NA	2.7×10^{-6}
TK64(KCBM1)	pB48	TK54(KCBM2)	6.3×10^{-6c}	1.9×10^{-6}
TK64(KCBM1)	pB91	TK54(KCBM2)	1.8×10^{-1}	3.8×10^{-6}

^a Number of plasmid-carrying *Spc*^r colonies relative to all *Spc*^r colonies.

^b Number of His⁺ *Spc*^r recombinant colonies relative to total colonies.

^c All 150 tested were Pro⁺ His⁻ Leu⁻.

^d NA, not applicable.

In summary, a novel *clt* locus has been identified in plasmid pJV1. Its unique sequence shows the presence of direct and inverted repeats, which is reminiscent of *oriT* regions of conjugative plasmids. The phenotype conferred on pJV1 derivatives by *clt* is similar, but not identical, to that of the analogous *clt* region of pIJ101.

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