Location of the genes for anthranilate synthase in *Streptomyces venezuelae* ISP5230: genetic mapping after integration of the cloned genes

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The anthranilate synthase (*trpEG*) genes in *Streptomyces venezuelae* ISP5230 were located by allowing a segregationally unstable plasmid carrying cloned *S. venezuelae* *trpEG* DNA and a thiostrepton resistance (*tsr*) marker to integrate into the chromosome. The integrated *tsr* was mapped by conjugation and transduction to a location close to *tyr-2*, between *arg-6* and *trpA13*. A genomic DNA fragment containing *trpC* from *S. venezuelae* ISP5230 was cloned by complementation of a *trpC* mutation in *Streptomyces lividans*. Evidence from restriction enzyme analysis of the cloned DNA fragments, from Southern hybridization using the cloned *trp* DNA as probes, and from cotransduction frequencies, placed *trpEG* at a distance of 12–45 kb from the *trpCBA* cluster. The overall arrangement of tryptophan biosynthesis genes in the *S. venezuelae* chromosome differs from that in other bacteria examined so far.

Introduction

The chromosomal organization of genes in the highly conserved tryptophan biosynthesis pathway of bacteria shows remarkable diversity; it varies from an operon-like arrangement in enteric species to a more dispersed pattern of individual genes or clusters of genes in most other groups (Crawford, 1975, 1980, 1989). In streptomycetes, which are soil bacteria belonging to the high-G + C subdivision of Gram-positive eubacteria (Woese, 1987), tryptophan biosynthesis (*trp*) genes are located on the chromosome in at least two clusters. Conjugational mapping in *Streptomyces coelicolor* A3(2) placed *trpD* (encoding phosphoribosyl transferase) and *trpF* (encoding phosphoribosyl isomerase) together at a location distinct from that at which *trpA* and *trpB* (encoding the α - and β -subunits, respectively, of tryptophan synthase) and *trpC* (encoding indolyglycerol phosphate synthase) were positioned (Smithers & Engel, 1974). A similar arrangement was demonstrated in *Streptomyces venezuelae*, where more precise mapping by SV1 phage-

mediated transduction established that *trpA*, *trpB* and *trpC* were cotransducible with each other as well as with neighbouring *his* genes. The group of genes is located within a 45 kb span of DNA (the approximate size of the phage SV1 genome); the gene order *hisA-hisB-trpC-trpB-trpA* was deduced from relative cotransduction frequencies (Stuttard, 1983; Paradkar *et al.*, 1991*b*). Also, a 4.2 kb genomic DNA fragment cloned from a third species, *Streptomyces griseus*, complemented *trpA*, *trpB* and *trpC* mutations in *Escherichia coli*, suggesting again that these genes are adjacent on the streptomycete chromosome (Rivero-Lezcano *et al.*, 1990). There is no comparable information on the organization of other tryptophan biosynthesis genes in streptomycetes; in particular, *trpE* and *trpG* (encoding the aminase and glutamine amidotransferase activities, respectively, of anthranilate synthase) have not been mapped.

Information about *trp* gene organization in bacteria can be incomplete because no genetic mapping system is available or, as in *S. venezuelae*, because some classes of mutations are absent from the collection of *trp* auxotrophs used for genetic analysis. To circumvent these difficulties, *trp*-containing DNA fragments from bacteria of interest have been cloned by complementation of a relevant *trp* auxotroph of *E. coli* (Brahamsha & Greenberg, 1987; Sato *et al.*, 1988; Sibold & Henriquet,

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Table 1. *Bacteria and plasmids used*

Strain	Genotype/phenotype	Source/reference
<i>Streptomyces venezuelae</i>		
ISP5230	Wild-type	E. Wellington, Warwick University, UK
AP41	VS194(pDQ189)	This study
AP52	VS194 with modified <i>trpEG</i>	Gene replacement (Paradkar, 1991)
VS161	<i>tyr-2 thrC1 uraA1</i>	Doull <i>et al.</i> (1986)
VS194	<i>hisA6 adeA10 SVP1⁻ SVP2⁻</i>	Doull <i>et al.</i> (1986)
VS309	<i>arg-6 thrC1 uraA1 adeA10 strA6</i>	C. Stuttard
VS420	<i>trpA13 pdx-4 cml-12</i>	Nitrosoguanidine mutagenesis of VS258 (Vats <i>et al.</i> , 1987)
VS255	<i>trpB14</i>	Nitrosoguanidine mutagenesis of ISP5230 (Paradkar <i>et al.</i> , 1991b)
<i>Streptomyces lividans</i>		
AP2	<i>trpC1</i>	Nitrosoguanidine mutagenesis of strain TK24 (Paradkar, 1991)
<i>Escherichia coli</i>		
TG1	<i>supE hsdΔ5 thi Δ(lac-proAB) F'(traD36 proAB⁺ lacI lacZΔM5)</i>	Carter <i>et al.</i> (1985)
Plasmids		
pDQ177	<i>tsr trpC</i>	This study
pDQ187	<i>amp trpEG</i>	Paradkar <i>et al.</i> (1991a)
pDQ189	<i>tsr amp trpEG</i> (bifunctional)	This study
pHJL400	<i>tsr, amp, lacZ'</i> (bifunctional)	Larson & Hersherberger (1986)
pIJ702	<i>tsr mel⁺</i>	Katz <i>et al.</i> (1983)

1988; Yelton & Peng, 1989). Function and sequence analysis of the cloned DNA and flanking regions has been used to detect the presence of other *trp* genes. This approach in *S. venezuelae* identified a 2.4 kb genomic DNA fragment that complemented an *E. coli trp* auxotroph lacking both TrpE and TrpG functions (Paradkar *et al.*, 1991a). We have now determined the chromosomal location of *trpEG* by mapping the thiostrepton resistance (*tsr*) marker of a segregationally unstable *trpEG*-containing recombinant vector that integrated into the chromosome of *S. venezuelae* by homologous recombination.

Methods

Cultures. Bacteria and plasmids used are listed in Table 1. *Streptomyces* were grown on MYM agar (Doull *et al.*, 1985) supplemented where necessary with thiostrepton (50 µg ml⁻¹). The *E. coli* strains were normally grown either on LB medium or on M9 (glucose/ammonium chloride/salts) medium (Sambrook *et al.*, 1989) supplemented with appropriate nutrients; for selecting transformants, ampicillin (100 µg ml⁻¹) was included.

Transformation procedures. Protoplasts of *S. venezuelae* were prepared and transformed with plasmid DNA as described by Aidoo *et al.* (1990). Transformants were selected by regeneration of protoplasts on R2YE medium (Hopwood *et al.*, 1985) modified by replacing sucrose and glucose with 0.3 M-sodium chloride and maltose, respectively. The agar surface was overlaid with soft nutrient agar containing sufficient thiostrepton to give an overall concentration of 25 µg ml⁻¹. Competent cells of *E. coli* auxotrophs were prepared by the procedure used by Kushner (1978) and were transformed with plasmid

DNA as described by Sambrook *et al.* (1989). Plasmid DNA was isolated by the alkaline lysis method of Kieser (Hopwood *et al.*, 1985).

Hybridization. Restriction endonuclease digests of genomic DNA were fractionated by electrophoresis in 0.7% agarose gels and were partially depurinated in 0.25 M-HCl for 20 min. The DNA was transferred to a nylon membrane under vacuum and probed with plasmid DNA labelled with [α -³²P]dCTP by the random primer extension method (Sambrook *et al.*, 1989). Hybridization was carried out at 65 °C for 14–16 h in a solution containing 20 × SSPE (3.6 M-NaCl, 0.2 M-sodium phosphate and 0.02 M-disodium EDTA); the membrane was washed at high stringency (0.1 × SSC at 65 °C for 10 min). The 1 × SSC solution contained 0.15 M-NaCl and 0.015 M-sodium citrate, pH 7.0.

Mapping by conjugation. Approximately 10⁶ spores from a strain carrying presumptive fertility plasmids SVP1 and SVP2 were mixed with excess (10–20-fold) spores from an SVP1⁻ SVP2⁻ strain and spread on nonselective (MYM) medium. After sporulation had occurred, progeny spores were plated on minimal medium supplemented to select one allele from each parent. Approximately 200 of the recombinant colonies that grew were replica-plated to diagnostic media to determine the frequency of each member of each pair of nonselected alleles present. The allele ratio of each new marker positioned it in the gradient of ratios from known markers on either side of the selected alleles; the position chosen was that which minimized the number of recombinants requiring multiple crossovers (Hopwood *et al.*, 1985; Doull *et al.*, 1986).

Mapping by transduction. A high-titre lysate of the donor phage SV1 (1 ml) containing 10¹⁰ p.f.u. ml⁻¹ was irradiated at 254 nm to 0.1% survival. A portion (50 µl) was spread with an equal volume of recipient spores (10⁸ c.f.u. ml⁻¹) on a medium that supported the growth of transductants but not the recipient genotype. Transductants were patched on a medium containing 10 mM-sodium citrate to prevent phage reinfection, and after sporulation were characterized by replica-plating on diagnostic media.

Fig. 1. Integration of the segregationally unstable vector pDQ189. (a) The vector was obtained by excising the cloned *S. venezuelae trpEG* from pDQ187 (Paradkar *et al.*, 1991a) as an *EcoRI*–*HindIII* fragment (bold line) and ligating it with pHJL400 (see Table 1) linearized by digestion with *EcoRI* and *HindIII*. Competent *E. coli* TG1 cells were incubated with the ligation mixture and transformants selected on ampicillin were screened on X-Gal and examined by gel electrophoresis for the presence of an 8.2 kb plasmid. (b) Distribution of *Bgl*II, *Cla*I and *Pst*I sites in the chromosomal DNA of *S. venezuelae* ISP5230 predicted from the sizes of restriction fragments hybridizing with ³²P-labelled pDQ189. (c) Sites generated by integration in tandem of 3–4 copies of pDQ189 by single reciprocal crossover(s) in the homologous *trpEG* region of the *S. venezuelae* chromosome. Bg, *Bgl*II; Ba, *Bam*HI; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; P, *Pst*I; Sa, *Sal*I; Ss, *Sst*I. *tsr*, *amp*, thiostrepton and ampicillin resistance genes, respectively. The numbers refer to restriction map distances in kb.

Results

Integration of pDQ189 into the *S. venezuelae* chromosome

The 2.4 kb region of *S. venezuelae* DNA containing *trpEG* was excised from pDQ187 (Paradkar *et al.*, 1991a) as an *EcoRI*–*HindIII* fragment and was ligated into the segregationally unstable vector pHJL400 (Larson &

Fig. 2. Southern hybridization using pDQ189 to probe restriction-enzyme-digested genomic DNA from *S. venezuelae* strains. (a) Separation of fragments by agarose gel electrophoresis; (b) autoradiogram of DNA fragments transferred from the gel to a nylon membrane and hybridized under stringent conditions with ³²P-labelled λ and pDQ189. Lanes: 1, *Hind*III digest of λ ; 2, *Kpn*I digest of λ (the *cos* ends of λ are annealed; the top band in the *Hind*III digest is 28 kb); 3, *Bgl*II digest of AP41; 4, *Bgl*II digest of VS194; 5, *Cla*I and *Pst*I double digest of AP41; 6, *Cla*I and *Pst*I double digest of VS194.

Hershberger, 1986) to create pDQ189 (Fig. 1a). To check the stabilities of pDQ189 and pHJL400, each plasmid was isolated from *E. coli* TG1 and used independently to transform *S. venezuelae* VS194 (*hisA6 adeA10 SVP1*–*SVP2*) to thiostrepton resistance. When the two types of thiostrepton-resistant (Thio^R) transformants were propagated on MYM agar without thiostrepton selection, VS194(pHJL400) segregated Thio^S progeny at an estimated frequency exceeding 98% of the c.f.u. obtained. In contrast, VS194(pDQ189) under the same conditions lost only 75% of its initially Thio^R resistant population. In the 25% remaining, the resistant phenotype was lost at only a slow rate during extended propagation on thiostrepton-free MYM agar. After a cycle of growth and sporulation under these latter conditions, more than 90% of the c.f.u. from individual colonies yielded Thio^R progeny. Retention of thio-

Fig. 3. Results from mapping the *tsr* site in *S. venezuelae* ISP5230 by conjugation using the allele gradient procedure. The numbers in parentheses are the frequencies with which progeny carrying each pair of nonselected alleles were present. In the crosses shown, AP41 (*hisA6 adeA10 tsr SVP1⁻ SVP2⁻*) was mated with: (a) and (b) VS161 (*tyr-2 thrC1 uraA1*); (c) VS420 (*pdx-4 trpA13*); (d) VS309 (*arg-6 thrC1 adeA10 uraA1 strA6*).

streptom resistance was attributed to integration of the plasmid into the chromosome by recombination between homologous regions containing *trpEG* DNA.

Southern analysis with pHJL400 as a probe showed no hybridization with the genomic DNA of VS194 nor with that of a VS194(pHJL400) transformant that had lost thiostrepton resistance after nonselective propagation. The genomic DNA of Thio^R VS194(pHJL400) transformants gave the hybridization signal expected for the free plasmid. In contrast, genomic DNA samples of nine independently isolated stable VS194(pDQ189) transformants showed hybridization signals in the region corresponding to high-molecular-mass chromosomal DNA (data not shown). To determine whether pDQ189 had integrated into the chromosome, genomic DNA samples of the parental strain VS194 and a stable VS194(pDQ189) transformant (strain AP41) were digested with restriction enzymes and probed with pDQ189. Since the 2.4 kb *trpEG* insert but not the vector portion

of pDQ189 hybridized with genomic DNA of VS194, and the insert DNA contained no recognition sites for *Bgl*II, *Cla*I or *Pst*I, probing of VS194 genomic DNA digested with these enzymes furnished a restriction map of the chromosomal region containing *trpEG*. From the sizes of the single hybridizing fragments in digests with *Bgl*II (10.5 kb; Fig. 2, lane 4), a mixture of *Cla*I and *Pst*I (7.8 kb; Fig. 2, lane 6), and a mixture of *Bgl*II and *Pst*I (3.2 kb; data not shown), the relationships in Fig. 1(b) were deduced.

The hybridizing fragments in genomic DNA from *S. venezuelae* strain AP41 differed in number and size from those in VS194 digests, as would be expected if pDQ189 had integrated into the chromosome via single reciprocal crossover(s). Since pDQ189 has no *Bgl*II sites, integration of a single copy (8.2 kb) into the homologous region of the 10.5 kb sequence between the two *Bgl*II sites in VS194 should have increased the size of this fragment to 18.7 kb. However, the *Bgl*II digest of AP41

Table 2. Cotransduction of *tsr* (AP41) with *tyr*⁺ (VS161) and *trpB*⁺ (VS525)

Cross*			Transductants	
Donor	Recipient	Medium	No. tested	No. Thio ^R
AP41	VS161	MM + Thr + Ura + His + Ade	352†	0
AP41	VS525	MM + His + Ade	531‡	11

* The markers in the strains were: AP41, *hisA6 adeA10 tsr*; VS161, *tyr-2 thrC1 uraA1*; VS525, *trpB14*.

† Tyr⁺ transductants were selected and screened for Thio^R and auxotrophic markers.

‡ Trp⁺ transductants were selected and screened for Thio^R and auxotrophic markers.

DNA showed a hybridizing fragment estimated by comparison with λ DNA size markers to be 35–40 kb (see Fig. 2b, lane 3). A DNA fragment of this size would accommodate three or four copies (10.5 + 24.6 or 32.8 = 35.1 or 43.3 kb) of pDQ189 integrated in tandem into the chromosome. In double digests with *Cla*I and *Pst*I where VS194 gave a single 7.8 kb hybridizing fragment, AP41 yielded four fragments, estimated to be 3.0, 3.5, 5.2 and 7.3 kb in size (see Fig. 2b, lane 5). Both *Cla*I and *Pst*I have single sites in the vector region of pDQ189; the 3.0 kb and 5.2 kb fragments thus account for the complete pDQ189 sequence (see Fig. 1a), and their higher signal intensities compared with those of the right and left junction fragments (3.5 kb and 7.3 kb, respectively) are consistent with tandem plasmid integration. Four copies of pDQ189 giving a ratio of four 5.2 kb to three 3.0 kb to one each of the 3.5 kb and 7.3 kb fragments (see Fig. 1c) would be consistent with the Southern blot appearance. Double digests with *Bgl*II and *Pst*I gave hybridizing fragments at 2.7, 8.2 and 8.7 kb (data not shown). Their sizes and signal intensities indicate that the 2.7 and 8.7 kb signals were from single-copy junction fragments whereas that at 8.2 kb arose from multiple copies of pDQ189 DNA.

Chromosomal location of *trpEG*

The presence of pDQ189 within the chromosome of AP41 marked the *trpEG* locus with *tsr* from the vector. To determine the location of *trpEG* relative to other chromosomal markers, *tsr* was mapped by conjugation. Strain AP41 (*hisA6 adeA10 tsr* SVP1⁺ SVP2⁺) was mated with various multiply marked SVP1⁺ SVP2⁺ strains, and the recombinant progeny were analysed by the single-selection procedure (Hopwood *et al.*, 1985). The data from four crosses all placed *tsr* very close to *tyr-2* in the short arc between *trpA13* and *arg-6* (Fig. 3). This location for *tyr-2* is consistent with its earlier map assignment between *hisA6* and *thrC1* (Chaliciopoulos, 1985; Vats *et al.*, 1987), and is comparable with that of *tyrA,B* in *S. coelicolor* A3(2) (Hopwood & Kieser, 1990). In crosses with VS161, which carries the *tyr-2* marker,

no segregation was observed between *tyr-2*⁺ and *tsr* of AP41; other markers in each cross segregated independently.

The integrated *tsr* marker was mapped more precisely by SV1 phage-mediated transduction. With AP41 as the donor strain and VS161 (*tyr-2*) as the recipient, no cotransduction of *tsr* and *tyr*⁺ was detected (Table 2). However, with the same donor and VS525 (*trpB14*) as the recipient, cotransduction of *tsr* and *trpB*⁺ was observed, albeit at low frequency. The possibility that the Thio^R colonies were spontaneous mutants was ruled out by a Southern analysis, which demonstrated that pHJL400 hybridized at high stringency with the genomic DNA of transductants (data not shown). Cotransduction of *tsr* and *trpB* implies a separation no greater than 45 kb (the approximate size of phage SV1 genomic DNA) on the *S. venezuelae* ISP5230 chromosome. As expected for *trpB*–*hisA* cotransduction (Stuttard, 1983), approximately 14% of the transductants (13 of 94) were His⁺, and served as an internal control in the experiment. Moreover, none of the His⁺ transductants was Thio^R, indicating that the *his* and *trp* loci in AP41 were not separated by *tsr*. Also, since all 11 Trp⁺ Thio^R transductants were His⁺, the relative order of markers on the chromosome was clearly *his*–*trp*–*tsr* rather than *tsr*–*his*–*trp*. Neither experiment gave any Ade⁺ recombinants, and all VS161 recombinants retained the Thr⁺ Ura⁺ phenotype; this was consistent with there being no spurious cotransduction of donor alleles.

Location of *trpEG* in relation to *trpCBA*

Genomic DNA of *S. venezuelae* was partially digested with *Mbo*I and ligated with *Bgl*II-digested pIJ702 DNA (Katz *et al.*, 1983). When the ligation mixture was used to transform *S. lividans* AP2 (*trpC*), a single prototrophic colony was found among 11000 Thio^R transformants. The plasmid (pDQ177) isolated from this transformant contained a 9.2 kb insert and efficiently transformed *S. lividans* AP2 to Thio^R prototrophs; in contrast to pIJ702, it hybridized at high stringency with *S. venezuelae* genomic DNA (Fig. 4a,b). Moreover, the number and

Fig. 4. (a) Separation of restriction-enzyme-digested genomic DNA fragments from *S. venezuelae* ISP5230 and AP52 by agarose gel electrophoresis. (b) Autoradiogram of DNA fragments transferred from the gel to a nylon membrane and hybridized with ^{32}P -labelled pDQ177 under stringent conditions. Lanes 1, λ digested with *HindIII*; 2, ISP5230 digested with *MluI*; 3, AP52 digested with *MluI*; 4, ISP5230 digested with *BglII*; 5, ISP5230 digested with both *ClaI* and *PstI*; 6, ISP5230 digested with *KpnI*. (c) Restriction map of the *trpC*-containing *S. venezuelae* DNA fragment cloned in pDQ177. The continuous line represents insert DNA; adjacent vector (pIJ702) DNA is dashed. The location of the *MluI* sites in the vector is uncertain. Bg, *BglII*; K, *KpnI*; M, *MluI*; Mb, *MboI*.

sizes of hybridizing fragments in restriction digests of *S. venezuelae* genomic DNA were consistent with the restriction map of the insert (Fig. 4c), indicating that a continuous piece of chromosomal DNA had been cloned. Detection of only one hybridizing fragment (28 kb) after double digestion with *ClaI* and *PstI* (see Fig. 4b, lane 5) confirmed that the insert had no site for either of these enzymes. The presence of two hybridizing fragments (6.8 and 16 kb) in the *BglII* digest of *S. venezuelae* genomic DNA (see Fig. 4b, lane 4) was consistent with the single site for this enzyme in the restriction map; likewise, the presence of 1.1, 1.2 and 10.5 kb hybridizing fragments in *KpnI* digests (see Fig. 4b, lane 6), and of fragments at 6.2, 4.6 and 3.2 kb in *MluI* digests (see Fig. 4b, lane 2) was compatible with the two restriction sites mapped for each of these enzymes.

Several lines of evidence indicated that *trpC* and *trpEG* are not adjacent. Restriction digests of the cloned DNA

containing these genes showed no fragments in common. In addition, Southern hybridization experiments showed that pDQ177 did not hybridize with pDQ181, the recombinant plasmid in which the 6.8 kb *trpEG*-carrying insert was initially cloned. The subcloned 2.4 kb insert containing *trpEG* DNA hybridized to *MluI* digests of *S. venezuelae* genomic DNA to give two fragments (12.0 and 16.5 kb), neither of which was among the hybridizing fragments obtained when *MluI* digests were probed with pDQ177 (containing the 9.2 kb *trpC* DNA). A strain (AP52) derived from VS194 by modifying a unique *MluI* site in the wild-type chromosomal *trpEG* sequence through an allele-exchange procedure gave the same three hybridizing fragments as wild-type *S. venezuelae* when samples of their genomic DNA were digested with *MluI* and probed with pDQ177 (see Fig. 4b, lane 3). Since strain AP52 carried the *trpEG* sequence on a single 28.5 kb *MluI* fragment (unpublished) the result shows that *trpC* does not lie in this segment of DNA, and thus is not within 12 kb on one side or 16.5 kb on the other side of *trpEG*.

Discussion

Attempts to clone genes in *S. venezuelae* ISP5230 by complementing host mutations have shown that recombinant plasmids carrying homologous *pab* (Aidoo *et al.*, 1990), *pdx* (Aidoo, 1989) or *trpD* (Paradkar, 1991) DNA integrate readily into the chromosome, and that the *tsr* marker of the integrated vector is cotransducible with neighbouring chromosomal markers. We have exploited this observation to determine the map location of *trpEG* by tagging its chromosomal site with the vector marker and subsequently mapping *tsr* by conjugation and transduction. The procedure is similar to that used to map the location of *IS110* insertion sequences (Chater *et al.*, 1985) and the 2.6 kb minicircle (Lydiat *et al.*, 1989) in *S. coelicolor* A3(2), and also to map the erythromycin resistance gene in *Saccharopolyspora erythraea* (Weber & Losick, 1988). The approach is particularly useful for chromosomal mapping of those genes for which mutant phenotypes are unavailable. It can be used advantageously in *S. venezuelae* ISP5230, where genetic mapping by transduction is well established (Stuttard, 1989).

Integration of vectors carrying homologous DNA inserts into streptomycete chromosomes can be achieved with *attP*-deleted ϕC31 phage (Chater & Bruton, 1983; Buttner *et al.*, 1990), the temperature-sensitive plasmid pGM2 (Muth *et al.*, 1989), nonreplicative *E. coli*-based vectors (Streicher *et al.*, 1989; Kuhstoss *et al.*, 1989; MacNeil *et al.*, 1992), single-stranded vectors (Hilleman *et al.*, 1991) and segregationally unstable plasmids (Larson & Hershberger, 1990). Among the

latter is pHJL400, a *Streptomyces*-*E. coli* shuttle vector containing the minimum replicon of the *S. coelicolor* plasmid SCP2* but lacking the *par* region encoding its partition function (Larson & Hershberger, 1986). That this vector might function in *S. venezuelae* ISP5230 was suggested by the earlier use of pDQ101, an SCP2* derivative with a deletion adjacent to the *par* region, to transform this strain (Aidoo *et al.*, 1990). Whereas pDQ101 was stable in *S. venezuelae*, pHJL400 was lost at a frequency comparable to that observed by Larson & Hershberger (1990) in *S. griseofuscus* transformants grown without thiostrepton selection. Stable transformants of *S. venezuelae* were readily isolated when the homologous *trpEG* DNA was present in a recombinant pHJL400 plasmid. Analysis of one such pDQ189 transformant showed 3–4 copies integrated in tandem at the *trpEG* site. Multiple tandem integration of plasmids carrying the 2.6 kb minicircle has been described in *S. coelicolor* A3(2) (Henderson *et al.*, 1989) and has been observed in *S. griseofuscus* using a vector of the same segregationally unstable type as pHJL400 (C. L. Hershberger, personal communication). Weber *et al.* (1990) found multiple copies of a recombinant pIJ702 plasmid integrated in the chromosome of *Sac. erythraea*.

The integrated *tsr* marker (and thus *trpEG*) was mapped unambiguously between *trpA13* and *arg-6*, close to *tyr-2*. A location for *trpEG* near, but not contiguous with, the *trpCBA* cluster was indicated by cotransduction at low frequency of *trpB*⁺ and *tsr*. The possibility that this event was due to two independent phage particles, one carrying *tsr* and the other carrying *trpB*⁺, infecting the same recipient can be excluded because, under optimum conditions, transduction of any given marker occurs at a frequency of only 10⁻⁶ p.f.u.⁻¹; co-infection by two transducing particles at a frequency of 10⁻¹² p.f.u.⁻¹ would be unlikely under the experimental conditions since no more than 10¹⁰ p.f.u. of phage were used for a transduction experiment. Moreover, if spurious cotransduction occurred under these conditions, it should also have been detected with the *tyr*⁺ allele. For cotransduction of *trpB*⁺ and *tsr* from the donor *S. venezuelae* strain AP41 containing tandemly integrated pDQ189, phage SVI must have packaged a DNA segment with regions capable of homologous recombination at each end of the complementing sequence. Complementarity at the *tsr* end might not require that the entire tandemly integrated plasmid and neighbouring chromosomal DNA be included in the phage. Since the cloned fragment of homologous DNA present in the plasmid was sufficient for integration, it should also provide the sequence required for a second reciprocal crossover during transduction. To possess the necessary sequence distal to *trpB*⁺, the packaged DNA need contain only one full copy of the integrated pDQ189

(to include *tsr*), and an adjacent additional copy of *trpEG* for terminus complementarity.

Analysis of the *S. venezuelae* DNA regions carrying *trpC* and *trpEG* indicated that they are separated by at least 12 kb. Location of *trpE* and *trpG* as a discrete pair separate from *trpC*, and thus from the *trpCBA* cluster (Stuttard, 1983; Paradkar *et al.*, 1991a), means that all tryptophan biosynthesis genes except *trpF* are now mapped in *S. venezuelae*. Auxotrophs carrying the *trpF* mutation are not yet available in this species. However, *trpF* has been mapped close to *trpD* in *S. coelicolor* A3(2), which so far shows a *trp* gene arrangement similar to that in *S. venezuelae* and *S. griseus* (Smithers & Engel, 1974). Because chromosomal maps of different species of streptomycetes show an overall conservation of linkage relationships (Stuttard, 1988), it is likely that, in most streptomycete species, the *trpCBA* cluster is at one location and *trpEG* forms a second locus; *trpD* and *trpF* are at a third location, but it is as yet unclear whether they are physically adjacent. In forming three distinct clusters, the streptomycete *trp* gene arrangement is similar to that in some other bacteria (Sawula & Crawford, 1972; Crawford, 1989; Johnston *et al.*, 1978; Bae *et al.*, 1989); however, the composition of the clusters differs. The *trp* gene arrangement in *S. venezuelae* is so far unique and contrasts with that in *Brevibacterium lactofermentum*, another member of the high G+C subdivision of Gram-positive bacteria, which has its *trp* genes in an operon similar to that of enteric bacteria (Matsui *et al.*, 1986).

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