Genetic Studies on SCP1-prime Strains of *Streptomyces coelicolor* A3(2)

By D. A. HOPWOOD AND HELEN M. WRIGHT

John Innes Institute, Colney Lane, Norwich NR4 7UH

(Received 10 December 1975)

SUMMARY

SCP1'-*cysB* carries a short chromosomal insertion: *cysB* was the only chromosomal locus identified on this plasmid; the plasmid was seldom lost; and a comparatively low frequency of crossing-over occurred between the exo- and endogenote in a *cysB*⁺/*cysB* heterozygote. SCP1'-*argA*, *uraB* carries a longer chromosomal insertion: five chromosomal loci have been detected on the plasmid; it was very often lost; and there was frequent crossing-over between exo- and endogenote in heterozygous strains.

Both SCP1' plasmids gave rise to plasmids closely resembling, or perhaps identical with, the original SCP1 by deletion of the chromosomal insertion. Markers *argA* and *uraB* were always deleted together from SCP1'-*argA*, *uraB*, whereas random deletion should more often have removed *uraB* alone. This suggests that special DNA sequences are involved in SCP1' formation.

An analysis of recombination between exo- and endogenote within a double heterozygote for *argA* and *uraB* supports the hypothesis that single copies of plasmid and chromosome assort from a pool of interacting molecules at, or immediately before, spore formation.

A strain carrying the SCP1'-*cysB* plasmid gave rise to a 'bidirectional' donor of chromosomal markers in which the plasmid had apparently integrated into the chromosome at or close to the *cysB* locus.

INTRODUCTION

Strains of *Streptomyces coelicolor* in which the SCP1 plasmid carries segments of the main chromosome have been named SCP1' by analogy with F' strains of *Escherichia coli* K12; they were isolated from SCP1⁺ (formerly called IF) strains (Hopwood & Wright, 1973*b*, 1976). When grown with an SCP1⁻(UF) strain, SCP1' cultures donate their plasmid to a very high proportion (30 to 90 %) of the SCP1⁻ population, just as SCP1⁺ strains donate the wild-type SCP1 plasmid very efficiently (Vivian, 1971; Puglia *et al.*, 1973).

Two SCP1' plasmids have been described: SCP1'-*cysB* and SCP1'-*argA*, *uraB*. Strains carrying SCP1'-*cysB* are relatively stable, losing the plasmid to yield SCP1⁻ derivatives spontaneously with a frequency of about 5 % (Hopwood & Wright, 1973*b*). In contrast, strains carrying SCP1'-*argA*, *uraB* are so unstable that a freshly cloned culture may contain 50 % or more of SCP1⁻ spores (Hopwood & Wright, 1974). Correlated with this difference, heterozygous CysB⁺ strains produced by transfer of the SCP1'-*cysB*⁺ plasmid into an SCP1⁻ *cysB* strain remain heterozygous for many subcultures, whereas heterozygous ArgA⁺ or UraB⁺ colonies from an SCP1'-*argA*⁺, *uraB*⁺ × SCP1⁻ cross rapidly become genotypically *argA*⁺ or *uraB*⁺ during subculture on minimal medium. It was postulated that the chromosomal insertion into the plasmid in the SCP1'-*argA*, *uraB* strain is long enough to allow frequent crossing-over with the chromosome, leading to integration of the exogenote

argA⁺ or *uraB*⁺ allele, whereas the insertion in the SCP1'-*cysB* plasmid is too short for frequent crossing-over. This postulate was supported by the finding that integration of *argA*⁺ or *uraB*⁺ was infrequent when the SCP1'-*argA*⁺, *uraB*⁺ plasmid was in *Streptomyces lividans* strain 66, whose chromosome is incompletely homologous with that of *S. coelicolor* A3(2) (Hopwood & Wright, 1973*a*). As well as giving rise to SCP1⁻ strains, both SCP1' strains were found to produce presumptive SCP1⁺ derivatives in which the plasmid had lost its chromosomal insertion by some kind of internal crossing-over or 'loop-out'.

The object of our experiments was to investigate in more detail the behaviour of the SCP1' plasmids. Mutations in chromosomal genes were followed to mark regions of the main chromosome or of insertions of it into SCP1' plasmids; production of, and resistance to, the SCP1-coded antibiotic (Vivian, 1971; Kirby, Wright & Hopwood, 1975, Wright & Hopwood, 1976) provided markers for the plasmid itself.

METHODS

General. Complete (CM) and minimal (MM) media and standard cultural and genetic techniques were those described by Hopwood (1967). CM was used for crosses, and MM, supplemented with growth factors required by auxotrophs, was used for other purposes. Cultures were incubated at 30 °C. Ultraviolet irradiation was done as described by Harold & Hopwood (1970), with a dose of approximately 3.8×10^{-4} J mm⁻², giving a survival of 1 to 5 %.

Starting strains. The strains, all derivatives of *Streptomyces coelicolor* A3(2), are listed in Table 1. The locations of the markers are shown on the linkage map in Fig. 1.

Construction of heterozygous strains and analysis of their segregation

SCP1'-cysB. A strain heterozygous for *cysB6* was made by crossing strain 1873, the original SCP1'-*cysB*⁺ isolate (Hopwood & Wright, 1973*b*), with SCP1⁻ strain 1956 and selecting for Cys⁺ phenotype on a medium containing histidine and streptomycin. For the experiment described here, a slant culture, freshly prepared from a single colony of this strain, was plated on non-selective medium and individual colonies were picked, 20 per dish, to plates of the same medium. The strains on these patch plates were analysed by replica plating as follows: to medium lacking cystine, to test for the presence of the *cysB*⁺ allele; to complete medium spread with a lawn of spores of SCP1⁻ strain 1190, inhibition of which indicated presence of the SCP1-coded antibiotic (Ant⁺ phenotype); and to complete medium spread with SCP1⁻ strain 1953 (*cysB6*), followed by replication of the resulting 'plate crosses' to minimal medium, to test for donation of the *cysB*⁺ allele.

SCP1'-argA, uraB. Strains heterozygous for one or more markers on the SCP1'-*argA*, *uraB* plasmid were unstable and had to be prepared immediately before analysis. The first such heterozygote was made by crossing strain 1928, the original SCP1'-*argA*⁺, *uraB*⁺ isolate (Hopwood & Wright, 1976), with SCP1⁻ strain A700 and selecting phenotypically Arg⁺ progeny on a medium supplemented with cystine and proline. Several individual colonies were streaked on non-selective medium and replicated to medium lacking arginine. The presence of some Arg⁻ colonies in such a streak confirmed heterozygosity of the original colony; one or more Arg⁺ colonies from the same streak were then grown on slants of non-selective medium and plated to give a population of derivative colonies. These were picked to patch plates and tested for Arg⁺ and Ant⁺ phenotype, and for donation of the *argA*⁺ allele to SCP1⁻ strain A702, or of the *uraB*⁺ allele to strain A663, by procedures analogous to those used to analyse derivatives of the SCP1'-*cysB* heterozygote. However,

Table 1. Starting strains of *Streptomyces coelicolor* A3(2)

All strains were from the John Innes Institute Culture Collection.

Strain no.	Chromosomal markers	Plasmid status
1098	<i>pheA1</i>	SCP1 ⁻
1190	<i>hisA1 uraA1 strA1</i>	SCP1 ⁻
1873	<i>pheA1</i>	SCP1 ⁻ - <i>cysB</i> ⁺
1945	<i>cysE24 pheA1</i>	SCP1 ⁻
1953	<i>cysB6</i>	SCP1 ⁻
1928	<i>pheA1</i>	SCP1 ⁻ - <i>argA</i> ⁺ , <i>uraB</i> ⁺
1956	<i>cysB6 hisD3 strA1</i>	SCP1 ⁻
2278	<i>argA1 cysD18 proA1</i>	SCP1 ⁺
2292	<i>argA1 uraB2</i>	SCP1 ⁻
2295	<i>argC4 hisA1</i>	SCP1 ⁻
2334	<i>argA1 uraB2 ureA32</i>	SCP1 ⁻
2398	<i>argA1 uraB2 genA3</i>	SCP1 ⁻
A236	<i>adeA13 pheA1 strA1</i>	SCP1 ⁻
A473	<i>argA1 proA1 uraA1 strA1</i>	SCP1 ⁻
A663	<i>uraB2</i>	SCP1 ⁻
A700	<i>argA1 cysD18 proA1</i>	SCP1 ⁻
A702	<i>argA1 metB4 pabA1 strA1</i>	SCP1 ⁻

Fig. 1. Linkage map of *Streptomyces coelicolor* A3(2) showing locations of the markers referred to in this paper.

because of the much greater instability of heterozygotes carrying the SCP1'-*argA*, *uraB* plasmid, it was possible to detect and classify heterogeneity within individual tested patches; auxotrophic areas in a prototrophic patch were readily seen by virtue of the slight leakiness of the markers, while SCP1⁻ areas in an otherwise Ant⁺ patch were detected by their partial inhibition, on the antibiotic indicator plates, by antibiotic produced by the SCP1' component of the patch.

Other heterozygotes for markers on the SCP1'-*argA*, *uraB* plasmid were made by crossing SCP1⁻ strains with SCP1' derivatives from the first and subsequent crosses in which recessive alleles had been transferred to the plasmid.

Table 2. *Possible derivatives from an SCP1' heterozygote*

Type	Constitution†	Class	Phenotype*		
			Aux ⁺ or Aux ⁻	Ant ⁺ or Ant ⁻	D or ND
1		SCP1'	Aux ⁺	Ant ⁺	D
2		SCP1'	Aux ⁻	Ant ⁺	ND
3		SCP1'	Aux ⁺	Ant ⁺	D
4		SCP1'	Aux ⁺	Ant ⁺	ND
5		SCP1 ⁻	Aux ⁻	Ant ⁻	ND
6		SCP1 ⁻	Aux ⁺	Ant ⁻	ND
7		SCP1 ⁺	Aux ⁻	Ant ⁺	ND
8		SCP1 ⁺	Aux ⁺	Ant ⁺	ND

* Aux⁺, prototrophic; Aux⁻, auxotrophic. Ant⁺, Antibiotic producer; Ant⁻, antibiotic non-producer. D, Donor of *a*⁺ allele to an SCP1⁻ *a*⁻ tester strain; ND, non-donor.

† —, Chromosomal material; , plasmid.

RESULTS

Possible derivatives from a heterozygous SCP1' strain

In principle, an SCP1' heterozygote could undergo several changes on propagation (Table 2). The original heterozygote is represented as type 1 in Table 2; crossing-over between homologous regions of exogenote and endogenote could result in either allele becoming homozygous (types 2 or 3), or in an exchange of alleles, with conservation of heterozygosity (type 4). Loss of the entire plasmid from any of types 1 to 4 would produce SCP1⁻ strains: type 5 from SCP1' types 1 and 2, and type 6 from types 3 and 4. Finally, 'loop-out' of the chromosomal insertion borne by the plasmid (or at least of that part of it carrying the marked locus) would produce strains of types 7 and 8.

Three phenotypic criteria would distinguish between various of the eight strain types: presence (Aux⁺) or absence (Aux⁻) of the prototrophic marker; production (Ant⁺) or non-production (Ant⁻) of the SCP1-coded antibiotic; and ability (D) or inability (ND) to donate the prototrophic exogenote marker to an SCP1⁻ tester strain. The phenotype of each strain is indicated in Table 2. These simple criteria do not distinguish between types 1 and 3, 2 and 7, or 4 and 8, but types 5 and 6 are defined uniquely. Further criteria, described below, were used to distinguish between phenotypically similar classes.

The possible occurrence of Ant⁻ strains by point-mutation of genes on the plasmid may be ignored in this analysis since such mutations are rare (Kirby, 1976).

Table 3. *Derivatives from an SCP1'-cysB⁺/cysB6 heterozygote*

Phenotype			Number	Type*
Cys ⁺ or Cys ⁻	Ant ⁺ or Ant ⁻	D or ND		
Cys ⁺	Ant ⁺	D	263	1 or 3
Cys ⁻	Ant ⁺	ND	10†	2 or 7
Cys ⁺	Ant ⁺	ND	2‡	4 or 8
Cys ⁻	Ant ⁻	ND	20	5
Cys ⁺	Ant ⁻	ND	2	6
Total			297	

D, Donor of *cys*⁺ allele; ND, non-donor.

* See Table 2.

† Subdivided into seven type 2, two type 7 and one 'bidirectional' chromosomal donor (see text).

‡ Subdivided into one type 4 and one type 8 (see text).

Table 4. *Stability tests on derivative strains from heterozygote SCP1'-cysB⁺/cysB6*

Strain type*	Treatment	Total no. of colonies	Ant ⁻ colonies	
			No.	Percentage
2	None	621	24	3.9
	u.v.	588	21	3.6
4	None	574	22	3.8
	u.v.	467	19	4.1
7	None	1178	1	0.1
	u.v.	1666	8	0.5
8	None	710	1	0.1
	u.v.	557	2	0.4

* See Table 2.

Derivatives from an SCP1'-cysB⁺/cysB6 heterozygote

A sample of 297 derivative colonies from an SCP1'-*cysB*⁺/*cysB6* heterozygote was analysed (Table 3). Twenty-two colonies (7.4 %) were Ant⁻ and therefore SCP1⁻ (types 5 and 6); of these, two were phenotypically Cys⁺ (type 6), indicating integration of the exogenate *cys*⁺ allele before plasmid loss. Of the Ant⁺ colonies, two failed to donate *cys*⁺ even though they contained this allele; evidently the *cys*⁺ allele had also been transferred to the endogenote in these two cases. It was possible to discriminate between a type 4 or type 8 constitution (Table 2) for these two colonies by the fact that SCP1'-*cysB* strains donate the chromosomal *pheA*⁺ marker to an SCP1⁻ strain with a higher frequency than do SCP1⁺ strains, even though this locus is not carried on the plasmid (Hopwood & Wright, 1973*b*); probably a transient interaction (donor crossing-over) between exo- and endogenote is responsible (Hopwood & Wright, 1976). In a 'plate-cross' test, a clear distinction was found between one strain (identified as type 4) that donated *pheA*⁺ to strain A236 with the same frequency as the original heterozygous culture, and the other (type 8) that had a negligible frequency of *pheA*⁺ donation, attributable to loss of the chromosomal region of the plasmid. The 10 Cys⁻ Ant⁺ colonies could similarly be subdivided into seven of type 2 and two of type 7, with one strain of an unexpected type. This turned out to be a donor able to transfer chromosomal markers near to and on either side of *cysB* with very high frequencies (see below).

Table 5. *Derivatives from an SCP1'-argA⁺/argA1 heterozygote*

Phenotypes			Number			Type*
Arg ⁺ or Arg ⁻	Ant ⁺ or Ant ⁻	D or ND	Clone A	Clone B	Clone C	
Arg ⁺	Ant ⁺	D	12	48	43	1 or 3
Arg ⁻	Ant ⁺	ND	0	2†	3†	2 or 7
Arg ⁺	Ant ⁻	ND	18	0	0	4 or 8
Arg ⁻	Ant ⁻	ND	0	5	14	5
Arg ⁺	Ant ⁻	ND	29	0	0	6
<i>Totals</i>			59	55	60	

D, Donor of *arg*⁺ allele; ND, non-donor.

* See Table 2.

† Subdivided into one type 2 and four type 7 (see text).

Table 6. *Stability tests on derivative strains from heterozygote SCP1'-argA⁺/argA1*

Strain type*	Treatment	Total no. of colonies	Ant ⁻ colonies	
			No.	Percentage
7†	None	1080	10	0.9
	u.v.	1173	27	1.2
7‡	None	1272	21	1.7
	u.v.	1280	27	2.1
8‡	None	1235	5	0.4
	u.v.	1277	32	2.5
8‡	None	1129	10	0.9
	u.v.	1024	13	1.3
SCP1 ⁺ control (strain 2278)	None	1450	1	0.1
	u.v.	1963	23	1.2

* See Table 2.

†, ‡ Replicate cultures of the same strain.

One example of each of strain types 2, 4, 7 and 8, recognized by the above criteria, was tested for the frequency with which it gave rise to SCP1⁻ derivatives on subculture, both spontaneously and after ultraviolet irradiation (Table 4). The type 2 and type 4 cultures showed a similar instability to that of the original SCP1^{-cysB} strain (Hopwood & Wright, 1973*b*), as expected on the hypothesis that they were structurally identical with it. The type 7 and type 8 cultures showed much lower instabilities, within the range for SCP1⁺ strains (Vivian & Hopwood, 1970), suggesting that they had lost the chromosomal insertion of the SCP1' plasmid and reverted to SCP1⁺.

No attempt was made to recognize an expected minority of type 3 strains amongst the 263 members of the Cys⁺ Ant⁺ donor class.

Derivatives from an SCP1'-argA⁺/argA1 heterozygote

A preliminary experiment was done with three Arg⁺ clones selected from a cross of strains 1928 and A700 (Table 5). There were no Arg⁻ segregants amongst 59 derivatives of clone A, suggesting that the starting colony for this clone had already lost the *arg*⁻ allele; probably it had become a type 3 strain. Clones B and C, on the other hand, contained Arg⁻ segregants, of both Ant⁺ and Ant⁻ phenotype, and doubtless originated from type 1 heterozygotes, as expected.

Table 7. *Derivatives from an SCP1'-arg⁺, ura⁺/argA1, uraB2 heterozygote*

Endogenote (chromosomal) genotype	Exogenote (SCP1') genotype					SCP1 ⁻ strains	'SCP1 ⁺ ' strains*
	<i>arg⁺ ura⁺</i>	<i>arg ura⁺</i>	<i>arg⁺ ura</i>	<i>arg ura</i>	Total		
<i>arg⁺ ura⁺</i>	0	0	0	1	1	0	0
<i>arg ura⁺</i>	17	0	12	2	31	26	0
<i>arg⁺ ura</i>	1	0	0	0	1	0	0
<i>arg ura</i>	78	3	26	9	116	63	2
Totals	96	3	38	12	149	89	2

* Type 7 strains (see Table 2).

Subdivision of the Arg⁻ Ant⁺ class of segregants into types 2 and 7 was readily done by testing for donation of *uraB⁺* to strain A663; type 2 strains donated *uraB⁺*, but type 7 did not. The two colonies from clone B and two of the three from clone C were found to be of type 7, the remaining colony being of type 2.

Stability tests (Table 6) on a type 7 culture, and on a type 8 culture from clone A, showed that they had regained stability, as had the comparable SCP1'-*cysB* derivatives described above. However, they had a slightly greater frequency of spontaneous plasmid loss than typical SCP1⁺ cultures (Vivian & Hopwood, 1970), including an SCP1⁺ derivative of A700 made for direct comparison (Table 6).

Derivatives from a double heterozygote involving the SCP1'-argA, uraB plasmid

A quantitative analysis was made of a double heterozygote of constitution SCP1'-*argA⁺, uraB⁺/argA1, uraB2* prepared by crossing strains 1928 and 2292. Three individual colonies from the cross were propagated and 80 derivative colonies from each clone analysed. Each of the 240 colonies was streaked in a patch, and phenotypic classification of the growth within the patch allowed the constitution of each colony to be deduced. The data from the three clones were very similar and were pooled to give an overall idea of events occurring during the propagation of the parental heterozygote.

The Ant⁻ (SCP1⁻) strains were characterized by simple replica plating; out of four possible genotypes, *arg ura* and *arg ura⁺* occurred in the sample analysed (Table 7). The Ant⁺ strains could, in principle, have included 16 genotypes: four combinations of exogenote markers with any one of the same four on the endogenote. Nine of these genotypes occurred (Table 7), with only about half (78) of the 149 strains retaining the genotype of the original heterozygote. The Ant⁺ strains were classified as follows. Within each patch, numerous SCP1⁻ areas were recognized, as described in Methods, and their genotype was determined by replication on media testing for Arg⁺ or Ura⁺ phenotype; the genotype of the majority class of such SCP1⁻ segregants defined the markers of the endogenote. Testing each patch for ability to donate *argA⁺* or *uraB⁺* might have indicated the presence or absence of these alleles on the exogenote. However, it transpired that any patch containing either or both of these alleles could donate them efficiently, even when the allele in question was on the endogenote and not on the exogenote, in the plating unit that gave rise to the colony being analysed. This occurred because crossing-over during the development of the colony exchanged endo- and exogenote markers in a significant proportion of the spores produced on that colony. Thus determination of the markers of the exogenote, when these were not defined automatically by the genotype of the endogenote and the phenotype of the patch as a whole, depended on the recognition of areas of auxotrophic Ant⁺ growth within the patch. For example, a patch that included prototrophic growth and contained *arg ura⁺* as the majority

SCP1⁻ component could have had the constitution SCP1⁻-*arg*⁺, *ura*⁺/*arg*, *ura*⁺ or SCP1⁻-*arg*⁺, *ura*/*arg*, *ura*⁺. The former showed no sign of the Ura⁻ phenotype, whereas the latter had significant areas of Ura⁻ generated by crossing-over within the colony under study. Two of the Ant⁺ patches, both of phenotype Arg⁻ Ura⁻, showed no sign of SCP1⁻ segregation and were classified as type 7 (Table 2) derivatives. Table 7 shows that the relative frequencies of the SCP1⁻ genotypes were not different from those of the endogenote genotypes in the SCP1⁺ strains (0.26:0.63 compared with 1:31:1:116), as would be expected if they had arisen by loss of the plasmid from a random sample of the SCP1⁺ derivatives.

The frequency distribution of the different SCP1⁺ genotypes can be explained in different ways, of which two extreme cases may be considered. One possibility is a 'group interaction' between a population of plasmids and a population of chromosomes followed by random assortment of plasmid and chromosomal genotypes from the mixture of interacting molecules, a situation having features in common with a bacteriophage cross. Alternatively, there might be exclusively pairwise interactions between plasmids and chromosomes, followed by segregation in discrete lineages. Since all except 13 of the 71 recombinant SCP1⁺ strains were homozygous for one or other allele at one or both loci, a pairwise interaction model with simple reciprocal crossing-over at a 'two-strand' stage is excluded and one would require a more complex model, with some non-reciprocal crossing-over (Herman, 1968; Berg & Gallant, 1971), and/or reciprocal crossing over at a 'four-strand' stage analogous to mitotic crossing-over in a eukaryote (Meselson, 1967).

An additional uncertainty is introduced by lack of critical evidence as to the origin of the chromosomal insertion into the SCP1⁺ plasmid. It is difficult to conceive of the origin of the plasmid within the SCP1⁺ culture except via a transient integration into the chromosome followed by 'loop-out'. Therefore the type I and type II F' models have to be considered, even though type I F' plasmids are much more numerous in *Escherichia coli* than type II (Low, 1972). In type I, the chromosomal insertion represents material from only one side of the integration site and is collinear with a corresponding segment of the main chromosome; in type II, material on both sides of the integration site is incorporated into the plasmid so that the insertion is made up of two adjacent sections of the main chromosome joined in reverse order. Clearly, the configurations formed in the SCP1⁺ strain by synapsis of exo- and endogenote would be different for the two alternatives, leading to different consequences for the various patterns of crossing-over. However, in the present example, unless the SCP1 plasmid had happened to integrate between *argA* and *uraB*, so that the two loci would have had to be picked up to form the SCP1⁺ from different sides of the integration site, it is unimportant whether a type I or type II origin was involved. A type I structure is therefore assumed.

The 'group interaction' and 'mitotic crossing-over' models lead to different predictions for the relative frequencies of certain recombinant SCP1⁺ genotypes. On the 'group' model, assuming a fairly large population of molecules, exo- and endogenote genotypes would be combined at random; on the 'mitotic crossing-over' model there should be a tendency for complementary genotypes to be associated. The present example is not ideal for a distinction between these two alternatives because the *argA* locus was apparently near one end of the chromosomal insertion into the SCP1⁺ plasmid (see below), making many genotypes very rare. Nevertheless, it does suggest that the frequencies of the larger classes of progeny do not depart significantly from random association: a G-test (Woolf, 1957) on the data for rows 2 and 4 of Table 7 has a value of 4.35, which is not significant at $P = 0.05$ even assuming only two degrees of freedom to allow for the inadequate numbers in column 2.

The frequencies of the exo- and endogenote genotypes from the SCP1⁺ strains in Table 7

Table 8. Analysis of the data of Table 7 in terms of the patterns of crossing-over required to generate individual *exo*- and *endogenote* genotypes

Parental constitution			
	<i>arg</i> ⁺	<i>ura</i> ⁺	
Intervals:	1	2	3
	<i>arg</i>	<i>ura</i>	
	Exo		
	Endo		
Genotype	Exogenote (SCP1')	Endogenote (chromosome)	Total
<i>arg</i> ⁺ <i>ura</i> ⁺	96 (n.c.o.)	1 (1,3)	97
<i>arg</i> <i>ura</i>	12 (1,3)	116 (n.c.o.)	128
<i>arg</i> ⁺ <i>ura</i>	38 (2,3)	1 (1,2)	39
<i>arg</i> <i>ura</i> ⁺	3 (1,2)	31 (2,3)	34

n.c.o., No crossing-over.

are analysed in Table 8. When the crossover patterns in the three intervals defined by the two loci and the two ends of the chromosomal insertion into the SCP1' plasmid are considered, complementary genotypes have very similar frequencies, except in one case. It is clear that interval 1 is short in comparison with the other two intervals. Taking the data at face value, and combining *exo*- and *endogenote* genotypes, estimates of the frequencies of double crossing-over in intervals 1,2:1,3:2,3 are 4:13:69. Solving the three equations gives the relative lengths of intervals 1:2:3 as 1:5:17.

Derivatives from a reciprocal double heterozygote involving SCP1'-argA, uraB

A simple way of considering part of the data in the previous section is to compute the relative frequencies, amongst recombinant SCP1' strains, of homozygosity for recessive alleles at either or both loci. The numbers were: five *Arg*⁻ *Ura*⁺, nine *Arg*⁻ *Ura*⁻ and 26 *Arg*⁺ *Ura*⁻. As a test of consistency, a partial analysis of a reciprocal heterozygote SCP1'-*argA1*, *uraB2/argA*⁺, *uraB*⁺, was carried out in which derivative colonies were classified solely on the basis of the gross phenotype of each patch. Out of 240 colonies, 14 (6 %) were SCP1⁻: 12 *arg*⁺ *ura*⁺ and two *arg*⁺ *ura*. It is significant that these represent the *endogenote* markers, respectively, of the parental heterozygote and of the commonest class of recombinant SCP1' strains, and are the complementary classes to those arising commonly from the reciprocal heterozygote. Of the 226 *Ant*⁺ colonies, 36 were auxotrophic for one or both markers: three *Arg*⁻ *Ura*⁺, six *Arg*⁻ *Ura*⁻ and 27 *Arg*⁺ *Ura*⁻, a ratio in good agreement with the results from the reverse coupling cross.

Loss of chromosomal markers from SCP1' plasmids

'Loop-out' of chromosomal markers to produce type 7 or type 8 strains (Table 2), closely resembling SCP1⁺ strains (Tables 4 and 6), occurred with a low but detectable frequency in the experiments already described. Three out of 275 (1 %) *Ant*⁺ derivatives of SCP1'-*cysB* (Table 3) were of this type. For SCP1'-*argA, uraB*, two out of 151 *Ant*⁺ derivatives in the experiment of Table 7 and four out of 96 *Ant*⁺ derivatives of clones B and C in the experiment of Table 5 were of type 7, making an average of 2 %.

In order to determine whether 'loop-out' of only part of the chromosomal insertion occurred with a significant frequency, a clone of strain 1928 was plated and the resulting

colonies were classified for antibiotic production and for ability to donate *argA*⁺ and/or *uraB*⁺ to strains A702 or A663 respectively. In two separate experiments, 22 out of 1113 (2.0 %) and 74 out of 2868 (2.6 %) Ant⁺ colonies lacked the ability to donate both *argA*⁺ and *uraB*⁺, while none (< 0.02 %) donated only one of the markers. Thus 'loop-out' of both loci was very much more common than loss of only one, if the latter occurred at all.

The limits of the chromosomal region carried by SCP1'-argA, uraB

Several loci have been mapped in the region of the chromosome close to *argA* and *uraB* (Hopwood *et al.*, 1973); of these, *cysE* was located between the other two loci (Hopwood, 1967). In a cross of an SCP1'-*argA*⁺, *uraB*⁺ strain with strain 1945, 67 % of the colonies selected on a medium supplemented with cystine and phenylalanine were phenotypically Cys⁺; all eight of a sample of these colonies gave rise to frequent Cys⁻ segregants. These results indicated that the *cysE* locus is borne on the SCP1'-*argA, uraB* plasmid, as predicted from its map location.

It was already reported (Hopwood & Wright, 1976) that the chromosomal insertion of the plasmid did not extend in an anticlockwise direction as far as *hisA*, and similar experiments indicated that the *uvrA, C, D* cluster was also outside the limits of the insertion. The anticlockwise limit of the insertion was defined by a study of mutation *argC4*. Loci *argA* and *argC*, mutations in which have a similar phenotype, were originally defined as separate loci by complementation tests using heteroclones (Hopwood, 1967). Strain 1928 was crossed with strain 2295 (*hisA1 argC4*) and spores were plated on media containing histidine and arginine or histidine alone. The frequency of Arg⁺ colonies was 1.8×10^{-4} and 6×10^{-4} in two experiments, a thousand times lower than expected for an SCP1'-linked marker. Moreover, none out of 20 of these Arg⁺ colonies showed a segregation of Arg⁻. The majority of the progeny of the parental *his arg* phenotype had acquired the Ant⁺ character, and most of these phenotypically Arg⁻ colonies donated the *argA*⁺ allele to strain A702. A functional *argC*⁺ allele was evidently not present on the SCP1'-*argA, uraB* plasmid. A sample of four SCP1'-*argA*⁺/*argC4* progeny of the cross, each Arg⁻ in gross phenotype, gave rise to a reproducible low frequency of Arg⁺ colonies on subculture. The simplest interpretation is that the SCP1'-*argA, uraB* insertion carries part of the *argC* gene, overlapping the *argC4* site, so that recombination between exo- and endogenote could generate a functional *argC*⁺ allele. Since *argA* and *argC* probably form, with *argB*, a cluster of related loci, this agrees with the recombination analysis which indicated a short segment of chromosome anticlockwise of *argA1* on the SCP1'-*argA, uraB* plasmid.

The clockwise limit of the insertion was not defined precisely, but two more loci were found to be plasmid-borne. The *ureA* locus was mapped to a position some distance clockwise of *argA* (Hopwood, 1965) and was subsequently shown to be clockwise of *uraB*. In a cross of strain 1928 with strain 2334 (*argA1 uraB2 ureA32*) the great majority of Ura⁺ recombinants were Ure⁺, and most of a sample of these segregated some Ure⁻ colonies on subculture. Evidently, *ureA* was borne on the SCP1'-*argA, uraB* plasmid. Another locus (*genA*), mutations in which confer a slightly enhanced level of resistance to gentamicin (approx. 0.3 µg ml⁻¹ compared with 0.1 µg ml⁻¹ for the wild type), has recently been mapped clockwise of *argA* (Hopwood & Wright, unpublished results). On crossing strain 1928 with strain 2398 (*argA1 uraB2 genA3*), some 80 % of Ura⁺ colonies were gentamicin-resistant, and several Ura⁺ colonies selected on gentamicin segregated a proportion of gentamicin-sensitive derivatives. Evidently *genA* was also borne on the SCP1'-*argA, uraB* plasmid; moreover, these results indicate some dominance of gentamicin-resistance over sensitivity.

Fig. 2. Percentage frequencies of inheritance of donor markers in crosses of a 'bidirectional' donor derived from an SCP1'-*cysB*⁺/*cysB*6 heterozygote with SCP1⁻ strains. ● indicates the inferred site of integration of the plasmid.

A 'bidirectional' chromosomal donor derived from SCP1'-*cysB*

As described above, one of the Cys⁻ Ant⁺ derivatives of the SCP1'-*cysB*⁺/*cysB*6 heterozygote was exceptional in having acquired the ability to donate chromosomal markers near to, and on either side of, *cysB* with very high frequencies. These markers had not, however, been transferred to the SCP1' plasmid since there was no sign of their heterozygosity in the progeny of crosses. The strain was at least as stable as an SCP1⁺ strain: two out of 1891 (0.1 %) colonies spontaneously lost the donor capacity and became Ant⁻; after u.v. irradiation, four out of 1320 (0.3 %) were found.

A series of crosses with SCP1⁻ strains A236, A473 and A702 were analysed non-selectively, with samples of approx. 200 progeny taken from each cross. The crosses were 'ultra-fertile' in that negligible proportions of progeny of either parental phenotype were produced. Almost 100 % of the progeny were Ant⁺ donors like the parent. The frequency of inheritance of donor alleles, represented by auxotrophic alleles of *cysB* and *hisD* and the prototrophic alleles of the other loci, was high on either side of a point between *adeA* and *cysB* and fell sharply with increasing distance in each direction (Fig. 2). The data indicate a similar model to that proposed for NF × SCP1⁻ crosses (Hopwood *et al.*, 1969, 1973), with (almost) obligate inheritance of a plasmid integrated between *adeA* and *cysB*. That the site of integration was clockwise of *cysB* but anticlockwise of *adeA* was indicated by the finding that alleles of *cysB* segregated independently of those of *adeA* and *pheA*, but not those of *metB*; in the latter case there was a highly significant shortage of the genotype *cys*⁺ *met*⁺ which arose by multiple crossing-over on the proposed model. The new strain was clearly a donor of the 'bidirectional' type reviewed by Hopwood & Wright (1976). It is the first example in which the plasmid interaction point is not in the 9 o'clock sector of the linkage map.

DISCUSSION

SCP₁ is the first conjugative plasmid outside the Gram-negative eubacteria to have been clearly defined in genetic terms. The existence and behaviour of SCP₁' strains are therefore relevant for comparative questions about bacterial plasmids, and indeed there are striking resemblances between SCP₁' strains of *S. coelicolor* and F' strains of *E. coli*.

The SCP₁' strains also facilitate genetic manipulations within *Streptomyces*. As an example, strains heterozygous for loci borne on SCP₁' plasmids frequently give rise to homozygotes for recessive alleles. These can be used as donors in further crosses with SCP₁⁻ strains bearing other mutations, thus allowing for complementation testing. Since crossing-over between the chromosomal insertion into an SCP₁' and the endogenote is frequent, a double heterozygote *in trans*, prepared by crossing a recessive homozygote for one locus with an SCP₁⁻ strain carrying a recessive allele at a closely-linked locus, gives rise to double recessive homozygotes with a significant frequency. By plasmid loss, such strains segregate double recessive SCP₁⁻ strains. Thus strains carrying recessive markers at closely-linked loci can be isolated simply. The potential value of SCP₁' strains in dominance testing has also been illustrated, by a cross involving a mutation in *genA*.

SCP₁' plasmids have a tendency to lose their chromosomal insertion, and a return to a structure closely resembling the wild-type SCP₁⁺ plasmid is more probable than a random loss of parts of the insertion. It is noteworthy that the 'loop-out' strains derived from SCP₁' have a similar degree of stability to SCP₁⁺. Moreover, the SCP₁'-*argA*, *uraB* plasmid was 100 times more likely to lose both chromosomal loci than only one, whereas, because of the relative distances of the two loci from the ends of the insertion, the *uraB* locus should have been lost several times more often than both loci together, assuming loss by some event proportional to distance. One might speculate that some kind of special sequences are involved in SCP₁' formation and dissociation, as in *E. coli*. It will be interesting to discover if, in an organism with an average G + C ratio (73 %) in its DNA very different from that of the Enterobacteriaceae (50 %), similar or identical sequences are involved (Cohen & Kopecko, 1975).

Further analysis, preferably of a double heterozygote involving the SCP₁'-*argA*, *uraB* plasmid but carrying markers more equally spaced will be needed to define more precisely recombination between SCP₁' plasmids and the chromosome. It is unlikely that this system will yield definitive information on the reciprocity of crossing-over: investigations of F' heterozygotes in *E. coli* (Meselson, 1967; Herman, 1968; Berg & Gallant, 1971) failed to provide a complete answer to this question. However, it should be possible to confirm or refute the indication from the present results that individual plating-units (very largely single spores) produced by a heterozygous SCP₁' culture contain single exo- and endogenotes chosen at random from a population of interacting plasmids and chromosomes. These data support the conclusions from different experiments (Kirby, 1976) that the number of copies of the SCP₁ plasmid per spore is low, if not unity. Apparently there is no large excess of heterozygous genotypes of parental type that have no chance of undergoing recombination. An opportunity for assortment of exo- and endogenotes from a considerable pool of molecules may be provided by the mycelial nature of *S. coelicolor*. Microscopy (Hopwood & Glauert, 1960*a, b*) showed long segments of hyphae, of both the substrate and young aerial mycelia, each apparently containing enough nuclear material to represent several chromosomes, which appeared to assort individually into spores.

Bidirectional donors of chromosomal markers have been isolated from unstable unidirectional donors (Vivian & Hopwood, 1973). The isolation of a bidirectional donor

from the well-characterized SCP1'-*cysB* is further evidence that unstable unidirectional donors are SCP1' strains carrying unmarked regions of the chromosome (Hopwood & Wright, 1976). The origin of an integrated donor by a single crossover between exo- and endogenote, followed by loss of one copy of the resulting chromosomal duplication, is plausible, but there is no satisfactory explanation for the bidirectional gradient of marker donation.

The present results bring the number of loci positively identified on the chromosomal insertion into the SCP1'-*argA*, *uraB* plasmid to five (*argA*, *cysE*, *uraB*, *ureA* and *genA*); the recombinational analysis of the double heterozygotes for *argA* and *uraB*, which indicated a much longer length of chromosome clockwise of *uraB* than anticlockwise of it, suggests that this is a considerable underestimate. This finding is part of the evidence that the plasmid DNA of molecular weight 18×10^6 to 20×10^6 , recently identified in SCP1⁺ and SCP1⁻ strains (Schrempf *et al.*, 1975), does not include SCP1. It was found (M. J. Bibb, unpublished results) that the molecular weight of plasmid DNA from an SCP1'-*argA*, *uraB* strain did not differ significantly from that of SCP1⁺ or SCP1⁻ strains. If SCP1 had been a molecule of molecular weight 18×10^6 to 20×10^6 , its mass would certainly have been increased to a detectable extent by a chromosomal insertion of the size carried by SCP1'-*argA*, *uraB*.

REFERENCES

- BERG, D. E. & GALLANT, J. A. (1971). Tests of reciprocity of crossing over in partially diploid F' strains of *Escherichia coli*. *Genetics* **68**, 457-472.
- COHEN, S. N. & KOPECKO, D. J. (1975). Structural evolution of bacterial plasmids: role of translocating genetic elements and DNA insertion sequences. *Federation Proceedings* (in the Press).
- HAROLD, R. J. & HOPWOOD, D. A. (1970). Ultraviolet-sensitive mutants of *Streptomyces coelicolor*. I. Phenotypic characterization. *Mutation Research* **10**, 427-438.
- HERMAN, R. (1968). Identification of recombinant chromosomes and F-merogenotes in merodiploids of *Escherichia coli*. *Journal of Bacteriology* **96**, 173-179.
- HOPWOOD, D. A. (1965). New data on the linkage map of *Streptomyces coelicolor*. *Genetical Research, Cambridge* **6**, 248-262.
- HOPWOOD, D. A. (1967). Genetic analysis and genome structure in *Streptomyces coelicolor*. *Bacteriological Reviews* **31**, 373-403.
- HOPWOOD, D. A. & GLAUERT, A. M. (1960a). Observations on the chromatinic bodies of *Streptomyces coelicolor*. *Journal of Biophysical and Biochemical Cytology* **8**, 257-265.
- HOPWOOD, D. A. & GLAUERT, A. M. (1960b). The fine structure of *Streptomyces coelicolor*. II. The nuclear material. *Journal of Biophysical and Biochemical Cytology* **8**, 267-278.
- HOPWOOD, D. A. & WRIGHT, H. M. (1973a). Transfer of a plasmid between *Streptomyces* species. *Journal of General Microbiology* **77**, 187-195.
- HOPWOOD, D. A. & WRIGHT, H. M. (1973b). A plasmid of *Streptomyces coelicolor* carrying a chromosomal locus and its inter-specific transfer. *Journal of General Microbiology* **79**, 331-342.
- HOPWOOD, D. A. & WRIGHT, H. M. (1976). Interactions of the plasmid SCP1 with the chromosome of *Streptomyces coelicolor* A3(2). *Second International Symposium on the Genetics of Industrial Micro-organisms*, pp. 607-619. Edited by K. D. Macdonald. London and New York: Academic Press.
- HOPWOOD, D. A., HAROLD, R. J., VIVIAN, A. & FERGUSON, H. M. (1969). A new kind of fertility variant in *Streptomyces coelicolor*. *Genetics* **62**, 461-477.
- HOPWOOD, D. A., CHATER, K. F., DOWDING, J. E. & VIVIAN, A. (1973). Advances in *Streptomyces coelicolor* genetics. *Bacteriological Reviews* **37**, 371-405.
- KIRBY, R. (1976). *Plasmid genetics of Streptomyces coelicolor* A3(2). Ph.D. thesis, University of East Anglia, Norwich.
- KIRBY, R., WRIGHT, L. F. & HOPWOOD, D. A. (1975). Plasmid determined antibiotic synthesis and resistance in *Streptomyces coelicolor*. *Nature, London* **254**, 265-267.
- LOW, K. B. (1972). *Escherichia coli* K12 F-prime factors, old and new. *Bacteriological Reviews* **36**, 587-607.
- MESELSON, M. (1967). Reciprocal recombination in prophage λ . *Journal of Cellular Physiology* **70** (Suppl. 1), 113-118.
- PUGLIA, A. M., SPADA-SERMONTI, I., BASILE, S., MISURACA, F. & SERMONTI, G. (1973). Infectious transfer of a fertility factor in *Streptomyces coelicolor*. *Genetical Research, Cambridge* **21**, 107-113.

- SCHREMPF, H., BUJARD, H., HOPWOOD, D. A. & GOEBEL, W. (1975). Isolation of covalently closed circular deoxyribonucleic acid from *Streptomyces coelicolor* A3(2). *Journal of Bacteriology* **121**, 416-421.
- VIVIAN, A. (1971). Genetic control of fertility in *Streptomyces coelicolor* A3(2): plasmid involvement in the interconversion of UF and IF strains. *Journal of General Microbiology* **69**, 353-364.
- VIVIAN, A. & HOPWOOD, D. A. (1970). Genetic control of fertility in *Streptomyces coelicolor* A3(2): the IF fertility type. *Journal of General Microbiology* **64**, 101-117.
- VIVIAN, A. & HOPWOOD, D. A. (1973). Genetic control of fertility in *Streptomyces coelicolor* A3(2): new kinds of donor strains. *Journal of General Microbiology* **76**, 147-162.
- WOOLF, B. (1957). The log likelihood ratio test (the G-test): methods and tables for tests of heterogeneity in contingency tests. *Annals of Human Genetics* **21**, 397-409.
- WRIGHT, L. F. & HOPWOOD, D. A. (1976). Identification of the antibiotic determined by the SCP1 plasmid of *Streptomyces coelicolor* A3(2). *Journal of General Microbiology* **95**, 96-106.