Genetic Mapping and Characterization of Novel Mutations which Suppress the Effect of a *relC* Mutation on Antibiotic Production in *Streptomyces coelicolor* A3(2)

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Streptomyces coelicolor A3(2) has been used frequently for both genetic analysis and gene cloning [1]. Antibiotic production by streptomycetes including *S. coelicolor* A3(2) is abolished by *rel* mutations such as *relA* and *relC* (affecting genes encoding ppGpp synthetase I and ribosomal L11 protein, respectively) which severely impair ppGpp synthesis upon nutrient starvation (briefly reviewed by OCHI *et al.*[2]). Previously, we reported that acquisition of certain streptomycin-resistance (*str*) mutations suppresses the deleterious effects of *relA* and *relC* mutations on actinorhodin and undecylprodigiosin production[2,3]. Although several of the *str* mutants contained a mutation within *rpsL* coding for ribosomal protein S12, other *str* mutants possessed wild-type *rpsL* genes. In the present study we attempted to locate the non-*rpsL str* mutations on the *S. coelicolor* chromosome by genetic mapping. We also found that *S. coelicolor* strain M145, frequently used for physiological studies, harbors a mutation (termed *sre*) which suppresses the impairment of antibiotic production found in a *relC* mutant. This paper describes the genetic analysis of these *str* and *sre* mutations.

S. coelicolor strains used are listed in Table 1. Strain KO-100 is a *relC* (=*rplK*) deletion mutant which was originally isolated from a collection of spontaneous thiostrepton-resistant mutants and lacks the ability to produce actinorhodin and undecylprodigiosin due to the deficiency in ppGpp accumulation[2]. Strain KO-211 contains a copy of the plasmid SCP1 integrated at 9 o'clock on the genetic map; such SCP1^{NF} strains exhibit high recombination frequencies with other *S. coelicolor* derivatives. Strains KO-239 (*rif-1*) and KO-240 (*cam-1*) confer resistance to 150 μg/ml of rifampicin and 100 μg/ml of chloramphenicol, respectively. Strains were grown at 30°C on R3 medium[3] or GYM medium[4]. Crosses and the analysis of data were carried out as described by HOPWOOD and CHATER[5]. Recombinants arose at a frequency of 10^{-3} among spores that had developed after 5 days of mixed culture on R3 agar medium supplemented

Table 1. *S. coelicolor* A3(2) strains used in this study.

Strain	Description	Source or reference
1147	Prototrophic wild-type (SCP1$^+$ SCP2$^+$)	HOPWOOD *et al.*[1]
M145	Prototroph (SCP1$^-$ SCP2$^-$) *sre-1*	HOPWOOD *et al.*[1]
M600	Prototroph (SCP1$^-$ SCP2$^-$)	CHAKRABURTTY and BIBB[8]
M570	*relA*	from M600 (CHAKRABURTTY and BIBB[8])
J1415	*proA1 argA1 cysD15 uraA1 nic tsp30* SCP1^{NF}	from K. CHATER
J1508	*uraA1 hisA1 strA1* SCP1^{NF} SCP2$^-$	from K. CHATER
KO-100	*relC* (=*rplK*)	from 1147[2]
KO-132	*relA str-1*	from M570[3]
KO-138	*relA str-5*	from M570[3]
KO-201	*uraA1 hisA1 strA1 relC* SCP1^{NF}	Conjugation between strains KO-100 and J1508 (this study)
KO-211	*proA1 argA1 uraA1 cysD18 nic relC* SCP1^{NF}	Conjugation between strains KO-201 and J1415 (this study)
KO-238	*relA relC str-1*	Conjugation between strains KO-132 and KO-211 (this study)
KO-239	*sre-1 rif-1*	Spontaneous rifampicin-resistant isolate from M145 (this study)
KO-240	*sre-1 cam-1*	Spontaneous chloramphenicol-resistant isolate from M145 (this study)
KO-258	*relC sre-1 cysD18*	Conjugation between strains M145 and KO-211 (this study)
KO-259	*relC sre-1 cysD18*	Conjugation between strains M145 and KO-211 (this study)

Table 2. Sensitivity of *S. coelicolor* strains to various antibiotics.

Strain	Minimum inhibitory concentration (MIC; μg/ml) of:							
	Streptomycin	Thiostrepton	Spectinomycin	Tetracycline	Lincomycin	Erythromycin	Chloramphenicol	Rifampicin
1147	1	2	30	10	20	10	30	10
M145 (*sre-1*)	1	2	30	10	20	10	30	10
M600	1	2	30	10	20	10	30	10
M570 (*relA*)	1	2	30	10	5	5	30	10
KO-132 (*relA str-1*)	5	2	10	5	5	5	5	10
KO-100 (*relC*)	1	200	30	1	10	5	15	10

with 100 μg/ml of each nutritional requirement. Thiostrepton was used to select for recombinants at a concentration of 20 μg/ml. The *nic* (requirement for nicotinamide) marker was not used for genetic analysis; nicotinamide was added to all media at a concentration of 10 μg/ml. Resistance to streptomycin was determined at a concentration of 5 μg/ml. Production of the pigment antibiotics actinorhodin and undecylprodigiosin was determined using R3 and GYM agar media supplemented with 100 μg/ml of each of the nutritional requirements. Experiments involving nutritional shift-down were carried out as described by OCHI[6]. Intracellular concentrations of ppGpp accumulated 15 minutes after shift-down were determined by high-pressure liquid chromatography as described earlier[4].

Characterization of *str-1* and *sre-1* Mutations

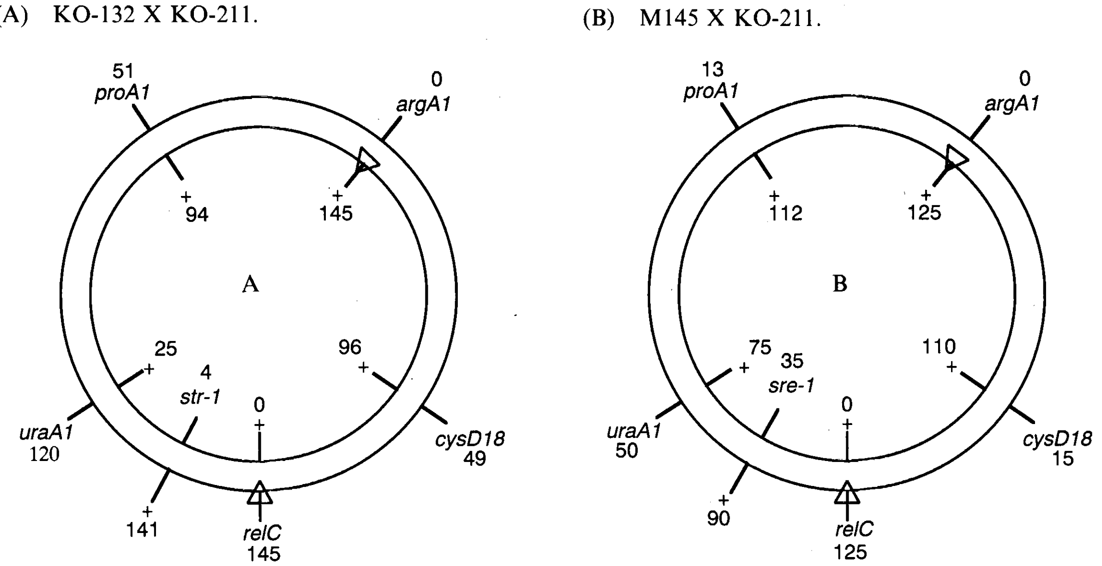
str-1 mutations that confer a low level of resistance to streptomycin (see Table 2) restore antibiotic production in the *relA* deletion mutant M570 without restoring ppGpp synthesis[3]. Although *str-1* mutants (strain KO-132) possessed wild-type *rpsL* genes, they showed increased sensitivity to chloramphenicol, spectinomycin, and tetracycline, whose antibiotic activities are attributed to inhibition of protein synthesis by ribosome binding. [The activities of the antibiotics listed in Table 2 are all attributed to the inhibition of protein synthesis, except for rifampicin whose activity is attributed to inhibition of RNA polymerase function.] These results support a previous suggestion[3] that the *str-1* mutation may reflect a lesion in a ribosomal protein other than S12 (or, if not, in one of the ribosomal RNAs). Strains M145 and M600 revealed unchanged sensitivity to each antibiotic tested when compared to the wild-type strain 1147, while strain M570 (containing a *relA* null mutant allele[7,8]) showed increased sensitivity to lincomycin and erythromycin (Table 2). The *relC* mutant KO-100 with a high level resistance to thiostrepton showed increased sensitivity to tetracycline (reference 2; and Table 2).

This *relC* mutant allele blocked antibiotic production when introduced into the wild-type strain 1147[2] and in strains M600, J1415 or J1508 (K. OCHI, unpublished work). However, antibiotic production in strain M145 was not blocked by introduction of the *relC* mutation. Apparently, strain M145 harbors a mutation(s) which suppresses the *relC* effect on antibiotic production. This mutation was designated *sre* (suppression of the *relC* effect). We next attempted to map the *sre* mutation, together with the *str-1* mutation, on the *S. coelicolor* chromosome. Unlike the *str-1* mutation, the *sre-1* mutation did not result in increased sensitivity to any of the antibiotics tested (Table 2).

Mapping of *str-1* and *sre-1*

In order to map the *str-1* mutation, a cross was performed between mutant KO-132 (*relA str-1*) and strain KO-211 (*proA1 argA1 uraA1 cysD18 nic relC* SCP1^{NF}). After selection for recombinants carrying *relC* (Thio^{R}) and *arg*$^{+}$, the other markers were scored (Fig. 1). All of the streptomycin-resistant (*str-1*) recombinants produced actinorhodin normally, while all of the streptomycin-sensitive recombinants failed to produce it. Analysis of allele frequencies indicated that *str-1* mapped close to *relC*. A position clockwise from *relC* is indicated by the apparent failure of *str-1* to segregate independently from *uraA1* (data not shown). Another *str* mutation (*str-5* of mutant KO-138) with a low level of resistance to streptomycin mapped to a similar position (data not shown). Thus, *str-1* and *str-5* mutations both locate to chromosomal positions distinct from that of *rpsL* (=*strA*) gene, which lies in an anticlockwise direction from *relC*[2,9].

Fig. 1. Genetic mapping of *str-1* and *sre-1*.



	ura^+	*uraA1*		cys^+	*cysD18*
str^+	21	120	str^+	92	49
str	4	0	*str*	4	0

	ura^+	*uraA1*		cys^+	*cysD18*
sre^+	40	50	sre^+	75	14
sre	35	0	*sre*	35	1

Strains KO-132 (*relA str-1*) and M145 (*sre-1*) [inner circle] were crossed with strain KO-211 (*proA1 argA1 uraA1 cysD18 nic relC*) [outer circle]. Selection was for recombinants carrying *relC* ($Thio^R$) and arg^+ (triangles). Numbers around the circles indicate allele frequencies among the recombinants scored. Segregation of *str* or *sre* with respect to *uraA* and *cysD* is tabulated.

Similarly, the *sre-1* mutation mapped close to, and in a clockwise direction from *relC* in a cross between M145 (*sre-1*) and KO-211 (Fig. 1). Selection was for *relC* ($Thio^R$) arg^+, and the *sre* mutation was detected by virtue of its ability to elicit actinorhodin production in a *relC* genetic background. A position clockwise from *relC* was indicated by the fact that actinorhodin-producing recombinants were found at a frequency of 66% (23/35) among the ura^+ *relC cysD* recombinants, while no actinorhodin-producing strain (0/23) was detected among the *uraA relC* cys^+ recombinants.

Since *str-1* and *sre-1* mapped very close to one another (Fig. 1), it is possible that these mutations are located within the same gene. To assess this possibility, crosses were performed between KO-239 (*sre-1 rif-1*) and KO-238 (*relA relC str-1*) or KO-240 (*sre-1 cam-1*) and KO-238. The ability of recombinants carrying *rif-1* and *relC* ($Thio^R$) or *cam-1* and *relC* ($Thio^R$), respectively, to produce actinorhodin was determined. More than 150 recombinants tested for each cross all produced actinorhodin normally, again suggesting that the *str-1* and *sre-1* mutations may be in the same gene. No accompanying restoration of ppGpp synthesis following nutritional shiftdown was detected in either the *relC str-1* or *relC sre-1* double mutant (<20 pmol/mg dry wt for strains KO-238, KO-258 and KO-259, compared with 230 pmol/mg dry wt for the wild-type strain 1147).

Concluding Remarks

In conclusion, the *str-1* mutation suppresses the effect of both *relA*[3] and *relC* (this study) on antibiotic production without restoring ppGpp synthesis, while *sre-1* suppresses the effect of *relC* (this study) but not *relA*. [Introduction of the *relA* mutation into strain M145 by allele replacement blocks antibiotic production[8].] The molecular basis for the *str-1* and *sre-1* mutations is unclear, but presumably these mutations, together with other *str* mutations giving rise to the altered ribosomal protein S12[3,10], exert their effect at the translational level. Our discovery of the *sre-1* mutation in strain M145 may account for the ability of this strain to produce greater amounts of actinorhodin than is produced by several other *S. coelicolor* strains tested (*e.g.* 1147, M600, J1415, J1508).

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