Genetic suppression analysis of non-antibiotic-producing mutants of the *Streptomyces coelicolor* *absA* locus

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The *absA* locus in *Streptomyces coelicolor* A3(2) was identified because mutations in it uncoupled sporulation from antibiotic synthesis: *absA* mutants failed to produce any of the four antibiotics characteristic of *S. coelicolor*. These mutants are now shown to contain point mutations in the *absA1* gene which encodes the histidine kinase sensor-transmitter protein of a two-component signalling system. The *absA1* non-antibiotic-producing mutants, which are unpigmented, spontaneously acquire pigmented colony sectors. Genetic analysis established that the pigmented sectors contain second-site suppressive mutations, *sab* (for suppressor of *abs*). Phenotypic characterization showed that *sab* strains produce all four antibiotics; some overproduce antibiotics and are designated Pha, for precocious hyperproduction of antibiotics. A set of *sab* mutations responsible for suppression was localized by plasmid-mediated and protoplast fusion mapping techniques to the vicinity of the *absA* locus. DNA cloned from this region was used to construct phage that could transduce *sab* mutations. Sequence analysis of *sab* strains defined *sab* mutations in both the *absA1* gene and the *absA2* gene; the latter encodes the two-component system's response regulator.

Keywords: *Streptomyces coelicolor*, antibiotic biosynthesis and regulation, two-component system, genetic suppression

INTRODUCTION

Actinomycetes produce most of the antibiotics useful for medical treatments and the genus *Streptomyces* contains an especially large number of antibiotic-producing species (Strohl, 1997). *Streptomyces coelicolor* has been the species of choice for genetic investigation of fundamental questions about antibiotic synthesis and regulation (Champness & Chater, 1994). Like other streptomycetes, *S. coelicolor* produces antibiotics in a growth-phase-dependent manner (reviewed by Bibb, 1996). In liquid cultures, antibiotics are produced only after the rapid growth phase. Antibiotic production is also

delayed in surface cultures, where it occurs in conjunction with sporulation.

In *S. coelicolor*, coupling of antibiotic production to sporulation is easily observed visually because two of the four antibiotics produced are pigments. Actinorhodin (Act) is blue or red, depending on pH, and undecylprodigiosin (Red) is red or yellow, its colour also varying with pH. Early genetic studies, using colour phenotypes to define the genes required for Act and Red synthesis (Rudd & Hopwood, 1979, 1980), showed that these genes comprised two clusters, *act* and *red*, respectively. The other two *S. coelicolor* antibiotics, methylenomycin (Mmy) and calcium-dependent antibiotic (CDA), have also been genetically characterized (Chater & Bruton, 1985; Chong *et al.*, 1998).

Molecular characterization of the antibiotic gene clusters has included cloning, sequencing, definition of transcripts and identification of cluster-specific regulatory genes. The *act* cluster consists of at least six transcripts and 20 ORFs. The *act*- and *red*-specific

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Abbreviations: Act, actinorhodin; CDA, calcium-dependent antibiotic; Mmy, methylenomycin; NF, normal fertility; Red, undecylprodigiosin; UF, ultra fertility.

regulators [ActII-ORF4 (Fernández-Moreno *et al.*, 1991; Gramajo *et al.*, 1993) and RedD (Takano *et al.*, 1992; Narva & Feitelson, 1990)] are the best understood. They are both activators with considerable amino acid sequence similarity and are members of the growing SARP family (streptomycete antibiotic regulatory protein) of transcriptional regulators (Wietzorrek & Bibb, 1997).

Growth-phase-regulated expression of these antibiotic-specific regulators is an important component of antibiotic regulation (Bibb, 1996). Approaches to defining the genetic mechanisms responsible (reviewed by Champness, 1999a) have included analysis of cloned genes that enhance antibiotic production, evaluation of the roles of metabolic regulators such as *relA* through gene disruption (e.g. Chakraborty & Bibb, 1997), and screening for mutations that perturb antibiotic regulation (reviewed by Champness, 1999b; Chater & Bibb, 1997).

In one genetic analysis of *S. coelicolor* antibiotic regulation, some mutations that blocked production of all four known antibiotics (Abs⁻ phenotype, for antibiotic synthesis deficient) were found to define the *absA* locus (Adamidis *et al.*, 1990). This locus was subsequently shown to encode a two-component signal transduction system (Brian *et al.*, 1996) composed of AbsA1, a homologue of 'orthodox' histidine kinase sensor-transmitter proteins, and AbsA2, a putative DNA-binding response regulator with the amino acid sequences conserved in 'orthodox' response-regulator proteins (Parkinson, 1995; Stock *et al.*, 1995). The *absA* locus regulates transcription of the *actII-ORF4* and *redD* pathway-specific activators (Aceti & Champness, 1998).

The *absA* mutants isolated on the basis of their Abs⁻ phenotype were shown, by marker rescue experiments (Brian *et al.*, 1996), to carry mutations of the *absA1* gene. In contrast, disruptions of the locus resulted in a phenotype of early onset enhanced antibiotic production. This phenotype was associated with disruption of both *absA1* and *absA2* or of *absA2* alone. Hence, we proposed (Brian *et al.*, 1996) that the *absA* locus exerts a negative regulatory effect on antibiotic production and that the Abs⁻ *absA* strains are mutationally locked into the negatively acting state.

Two Abs⁻ *absA* mutant strains, C542 and C577, were phenotypically characterized in detail. One shared trait is spontaneous apparent reversion. We were interested in determining the genetic events underlying formation of the apparent revertants because if these strains contained suppressive mutations, the suppressors might provide insights into the mechanisms of AbsA1/AbsA2 function or identify other genetic elements functioning in the *absA* pathway.

Here, we present a genetic analysis of the *absA* pseudoreversion phenomenon. We first define the *absA1* mutational alterations in Abs⁻ strains and then demonstrate that a set of pseudorevertant strains carries

second-site suppressive mutations, *sab* (for suppressor of *absA*). We use plasmid-mediated and protoplast fusion mapping techniques to locate *sab* alleles and then use a specialized transducing phage to show that some *sab* mutations map to the *absA* locus. By sequence analysis, we define the mutational alterations to the AbsA1 and AbsA2 proteins.

METHODS

Bacterial and phage strains. Derivatives of *S. coelicolor* A3(2) and phage strains are listed in Table 1. *Streptomyces lividans* 1326 was used for phage propagation. *Escherichia coli* DH5 α (Gibco-BRL) was used for plasmid cloning. *S. coelicolor* and *E. coli* culture techniques were as previously described (Brian *et al.*, 1996).

Culture conditions for antibiotic assays. R5 agar medium (Hopwood *et al.*, 1985) was used for characterization of Act, Red and Mmy. SMMS agar (Takano *et al.*, 1992) was also used for Act and Red, which were assayed as described previously (Brian *et al.*, 1996). Mmy assays were as described by Brian *et al.* (1996). For assay of CDA, low-calcium nutrient agar (Oxoid) with or without added calcium [as Ca(NO₃)₂ to 12 mM] was used (Adamidis *et al.*, 1990).

Isolation of *sab* strains. Pigmented strains were cultured from spore to spore a minimum of three times and then acid-treated (0.01 M HCl, pH 2.0, for 10 min), followed by neutralization with an equal volume of 0.01 M NaOH to kill any contaminating mycelia that might carry multiple genomes, and therefore carry a *sab*⁺ allele.

sab strains were isolated from spontaneously arising pigmented sectors in plates of strains C542 and C577. To ensure that independent *sab* mutations were isolated, Abs⁻ spores were plated and the resulting individual colonies were cultured through several rounds of streaking before pigmented sectors were selected. Each *sab* strain was isolated from a different Abs⁻ progenitor colony.

Genetic mapping techniques. Crosses and data analysis were carried out as described previously (Champness, 1988). For plasmid-mediated mating, chromosomal recombination was mediated primarily by plasmid SCP1 integrated at 9 o'clock on the genetic map to give the NF type (Hopwood & Chater, 1974). In an NF $\times$ SCP1⁻ cross (also referred to as NF $\times$ UF), close to 100% of the progeny will be NF (Hopwood *et al.*, 1969). Several phenotypes are associated with the NF state: (1) NF strains are Aga⁻ and fail to sink into the agar whereas Aga⁺ strains sink (Hodgson & Chater, 1981); and (2) NF strains (including Abs⁻ strains) are usually Mmy-resistant due to the presence of the *mmy* gene cluster on SCP1 (Kirby & Hopwood, 1977).

The frequencies of markers donated by the NF parent decrease in both clockwise and anticlockwise directions from the SCP1 insertion region at 9 o'clock (Hopwood *et al.*, 1985). If the SCP1⁻ parent is J1501 (Table 1), the *hisA1* and *strA1* alleles will be present in >95% of spore progeny plated non-selectively.

Protoplast fusions. PEG-mediated protoplast fusions were performed as described by Hopwood *et al.* (1985). Spores from regenerated protoplasts were plated on selective media.

Actinophage transductions. Transductions were carried out essentially as described by Piret & Chater (1985). Briefly,

Table 1. *S. coelicolor* A3(2) strains and *Streptomyces* phage

Strain/phage	Relevant characteristics	Source or reference
Strains		
J1501	<i>hisA1 uraA1 strA1</i> SCP1 ⁻ SCP2 ⁻ Pgl ⁻	Hopwood <i>et al.</i> (1985)
J650	<i>cysD18 mthB2</i> SCP1 _{NF} SCP2*	Hopwood <i>et al.</i> (1985)
1514	<i>cysA15 proA1 argA1 uraA1 nicA1</i> SCP1 _{NF} SCP2*	Hopwood <i>et al.</i> (1985)
TK16	<i>argA1 guaA1 redA59 act-117</i> (IV)	Hopwood <i>et al.</i> (1985)
TK18	<i>argA1 uraA1 strA1 redE60 act-141</i> (III)	Hopwood <i>et al.</i> (1985)
C420	Δ <i>absA1::ermE hisA1 uraA1 strA1</i> SCP1 ⁻ SCP2 ⁻ Pgl ⁻	Brian <i>et al.</i> (1996)
C430	Δ <i>absA2::RS500 hisA1 uraA1 strA1</i> SCP1 ⁻ SCP2 ⁻ Pgl ⁻	Brian <i>et al.</i> (1996)
M138	<i>argA1 cysD18 proA1</i> SCP1 _{NF}	Hopwood <i>et al.</i> (1985)
C542	<i>hisA1 uraA1 strA1 absA1-542</i> SCP1 ⁻ SCP2 ⁻ Pgl ⁻	Adamidis <i>et al.</i> (1990)
C577	<i>hisA1 uraA1 strA1 absA1-577</i> SCP1 ⁻ SCP2 ⁻ Pgl ⁻	Adamidis <i>et al.</i> (1990)
C5422	<i>cysA15 proA1 argA1 uraA1 absA1-542</i> SCP1 _{NF} SCP2*	Adamidis <i>et al.</i> (1990)
C5423	<i>cysD18 mthB2 absA1-542</i> SCP1 _{NF} SCP2*	This work†
C5422S1	C5422 <i>sab1</i> , Type II	This work
C542S2	C542 <i>sab2</i> , Type II	This work
C542S3	C542 <i>sab3</i> , Type I	This work
C542S9	C542 <i>sab9</i> , Type II	This work
C542S10	C542 <i>sab10</i> , Type I	This work
C542S11	C542 <i>sab11</i> , Type II	This work
C542S12	C542 <i>sab12</i> , Type II	This work
C577S20	C577 <i>sab20</i> , Type I	This work
C577S25	C577 <i>sab25</i> , Type II	This work
C577S27	C577 <i>sab27</i> , Type I	This work
C577S28	C577 <i>sab28</i> , Type II	This work
Phage		
KC516	ϕ C31-derived actinophage, Thio ^r att ⁻ c ⁺	Rodicio <i>et al.</i> (1985)
RS100	KC516 carrying 3.2 kb <i>XhoI</i> insert	Brian <i>et al.</i> (1996)
RS120	KC516 carrying <i>absA1-542</i> mutant 3.2 kb <i>XhoI</i> insert	Brian <i>et al.</i> (1996)
RS500	KC516 carrying 1.45 kb <i>XhoI</i> – <i>Bam</i> HI insert	Brian <i>et al.</i> (1996)

† Recombinant from cross of C542 × J650 strains.

phage suspensions were mixed with spores of the recipient strain on R5 agar. After colonies had sporulated, they were replicated to R5 agar containing thiostrepton (50 µg ml⁻¹) to select lysogens. Phage released from lysogens were isolated as plaques formed in *S. lividans* soft agar overlays poured on to 1-d-old lysogenic cultures streaked on nutrient agar. Nutrient agar and phage propagation techniques were those described by Hopwood *et al.* (1985).

DNA sequencing of *absA1* and *sab* mutant alleles. The *absA1* alleles of Abs⁻ strains C542 and C577 and the presumptive *sab* mutations were sequenced from nested PCR-generated fragments. The *absA1* gene (Brian *et al.*, 1996) was amplified from primers A15a (5'-CATCTGGCGGCGATCGGCAACGACCG-3'; nt 54–71 upstream of the *absA1* translation start site) and A13e (5'-CGCGAATCATCCGATCGTTCCTGGTG-3'; nt 2–28 downstream of the *absA1* stop codon). The PCR reaction mixtures (100 µl) contained 200 ng genomic DNA template, 0.3 µmol of each primer and 2.5 U *Pfu* polymerase (Stratagene). Template denaturation for 5 min at 95 °C was followed by 30 cycles of 95 °C for 1 min, 65 °C for 1.5 min and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The 1.8 kb *absA1* product was purified by agarose gel electrophoresis, digested with *Bam*HI and *Xho*I, and cloned in pBluescript II SK(+) (Stratagene). Because previous genetic mapping had localized the *absA1* mutations to the 1.45 kb *Xho*I–*Bam*HI segment of *absA1* (Brian *et al.*, 1996), sequence

analysis was restricted to this region. The end regions were sequenced from standard vector primers, while the internal region was sequenced from primer A15c (5'-CGCTACATCGCCGACCAG-3'; nt 546–563 of *absA1*) (Iowa State University DNA Sequencing Facility, Ames, IA, USA). The *absA1* *Xho*I/*Bam*HI regions of *sab* mutants C542S3 and C542S11 were sequenced in the same manner. In addition, the N- and C-termini of these alleles were confirmed by direct sequencing of the PCR product using primers A15a and A13e.

The *sab absA2* sequences were generated with primers A25a (5'-CAGGGAAGGATCCGATGATTGCG-3'; nt –18 to +10 of *absA2*) and A23a (5'-GGCGACCGGCGGATCCGCCTC-3'; nt 80–101 downstream of the *absA2* stop codon), both of which contain *Bam*HI restriction sites. Each reaction mixture (100 µl) contained 200 ng genomic DNA template, 0.3 µmol of each primer and 2.5 U *Taq* polymerase (Gibco-BRL). Samples were denatured at 95 °C for 5 min followed by 30 cycles of 95 °C for 1 min, 65 °C for 1.5 min and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The 0.8 kb product was purified on a Qiagen PCR purification column, digested with *Bam*HI, purified again and cloned in pBluescript II SK(+). Convergent overlapping sequences were obtained from standard primers contained on the vector. All mutations were verified by sequencing at least two independently generated PCR products. Sequences were analysed using the Wisconsin GCG package.

RESULTS AND DISCUSSION

Isolation and phenotypic characterization of revertant strains

The Abs⁻ phenotype of the *absA* mutant strains C542 and C577 is characterized by a failure to produce any of the known *S. coelicolor* antibiotics. Cultures grown on R5 agar therefore lack the blue, purple or brick (depending on pH) coloration typical of the parental J1501, but nevertheless form a sporulating aerial mycelium much like that of J1501.

While culturing Abs⁻ strains, we observed that both C542 and C577 appeared to revert spontaneously, acquiring pigmented sectors that were stably culturable. These could be sorted into two phenotypic groups: Type I produced a colony very similar to that of the J1501 parent in both pigmentation and morphology; Type II produced a colony that was very deeply pigmented and also was altered in morphology, with a crenulated surface and few aerial hyphae. The crenulation was especially noticeable in colonies grown at low density. Both C542 and C577 produced the two types of pigmented strains.

The frequency of phenotypic reversion in C542 and C577, although not quantified, appeared to be much higher than for other *S. coelicolor* non-antibiotic-producing mutants, including *absB*, *bldB*, *bldG*, *bldH* (W. Champness, unpublished) and *bldA* (Guthrie *et al.*, 1998; W. Champness, unpublished). By 2 weeks, many *absA* mutant colonies contained a pigmented sector, whereas cultures of the latter set of mutants typically contained only a few pigmented sectors per plate. Moreover, the pigmented sectors that arose spontaneously in *absB* and *bld* mutants were mostly of the Cm^s (chloramphenicol-sensitive), 'scarlet' type (Sermonti *et al.*, 1977), a pigmentation phenotype associated with DNA rearrangements (Dyson & Schrepf, 1987) but otherwise poorly understood. In contrast, the apparent revertant strains isolated from C542 and C577 were as Cm^r as J1501 (data not shown).

Four Type I and seven Type II pigmented strains (Table 1), all independently isolated, were evaluated in plate-grown cultures for production of the *S. coelicolor* antibiotics Act and Red. Each strain produced both antibiotics. All strains except C577S20 and C577S25 produced the CDA. An SCP1_{NF} strain, C542S1, produced Mmy. A notable characteristic of the Type II strains was that they produced Act (especially γ -actinorhodin; Bystrykh *et al.*, 1996) and Red about 1 d earlier than did J1501. Below we refer to this Type II phenotype as Pha (precocious hyperproduction of antibiotics).

Genetic evidence for second-site suppressor mutations

For initial genetic characterization, Pha Type II strain C542S11 was chosen; spores were purified by acid treatment (see Methods) designed to kill any mycelial

(multi-genomic) fragments and ensure genetic homogeneity.

To distinguish whether restoration of antibiotic production was due to a reverting mutation or to a second-site suppressing mutation, strain C542S11 was crossed to a standard mapping strain, J650, and progeny were examined for the Abs⁻ phenotype, occurrence of which would indicate that C542S11 carried both the *absA1-542* mutant site and a suppressive mutant site, *sab* (for suppressor of *abs*). Abs⁻ colonies were obtained at a frequency of about one in 5000 among Str^r progeny from the cross. In contrast, Abs⁻ colonies could not be found in a screen of approximately 200 000 colonies plated from C542S11 spores. Together, these results suggested that the Abs⁻ colonies arising from the C542S11 $\times$ J650 cross were likely *absA1-542 sab*⁺ recombinants. To verify that the recombinants' Abs⁻ phenotypes were due to *absA* mutant alleles, several Abs⁻ strains from the C542S11 $\times$ J650 cross were characterized in detail: their phenotypes were identical to that of C542, and genetic crosses demonstrated that each carried a mutant *absA* allele (data not shown).

Genetic mapping of *sab* suppressor mutations

The recovery of Abs⁻ recombinants from the C542S11 $\times$ J650 cross was strong evidence for the existence of a *sab* mutant allele in C542S11, and the very low frequency of Abs⁻ recombinants from the cross suggested that the *sab* mutation site was very close to the *absA1-542* mutant site. To map the putative *sab* locus, additional crosses were performed.

(i) Evidence consistent with a *sab* locus in the 10 o'clock region, where *absA* is also located, came from crossing strain C542S1 with C542 (Table 1). The results are shown in Fig. 1(a). C542S1 was a Type II *sab* mutant isolated in a genetic background (C5422 in Table 1) useful for mapping in the vicinity of *absA*; this strain had the genotype *sab absA1-542 cysA15 proA1 argA1 strA1 SCP1_{NF}*. Strain C542S1 carried SCP1 integrated in the 9 o'clock region, whereas C542 lacked SCP1. The fertility characteristics of this type of cross, referred to as 'NF $\times$ UF' (Hopwood & Chater, 1974), result in a curious bias in the alleles that are recovered in progeny spores, even in the absence of selection: all progeny inherit the integrated SCP1 plasmid but almost all carry genomic markers from the SCP1⁻ parent for the region extending clockwise from approximately 11 o'clock to about 7 o'clock. In Fig. 1(a), this effect can be observed for the *hisA* and *argA* loci: 66/68 spores were *hisA1 argA*⁺. Because 34/68 spores were Pgm⁺ (e.g. *sab* mutant) and the other 34 were Pgm⁻ (e.g. *sab*⁺), the *sab* locus appeared to be close to SCP1_{NF}, either between SCP1_{NF} and *cysA* or between SCP1_{NF} and *uraA*. Four additional *sab* mutants isolated from strain C5422 gave similar results.

(ii) The cross shown in Fig. 1(b) further refined mapping of the *sab* locus to the 10 o'clock region by assessing its linkage to *cysA*, a marker to which the *absA* locus is linked (Adamidis *et al.*, 1990). When strains C542S1

Fig. 1. (a) Cross of strain C5422S1 to parental strain C542. Recombinants were obtained from nonselective plating conditions (see Methods). Allele frequencies among the progeny are indicated. (b) Cross of pseudorevertant strain C5422S1 to strain C5423. Recombinants were selected as indicated by triangles. Allele frequencies among the recombinants are indicated. Segregation of Pgm⁺ and Pgm⁻ indicating the *sab* mutant and *sab*⁺ alleles, respectively, is tabulated.

Fig. 2. Mapping *sab* alleles. (a) Strain J650 was crossed with the *sab* strains listed. The percentage of recombinants that were Abs⁻ is tabulated for two selections. In selection (1) Str^r recombinants were selected (indicated by triangles); SCP1_{NF} is also inherited by most progeny (see Methods). In selection (2) His⁺ Str^r recombinants were selected (indicated by circles). Again, most progeny inherited SCP1_{NF}. (b) Protoplast fusion of strains J650 and *sab* strains C542S3 and C542S9. The percentage of recombinants that were Abs⁻ is tabulated for two selections. In selection (1), Ura⁺ Str^r recombinants were selected (indicated by triangles); in selection (2), His⁺ Str^r recombinants were selected (indicated by circles).

and C5423 (Table 1) were crossed, the *sab* allele segregated with the *cysA15* allele and lay anticlockwise to it, as had been previously observed for *absA1-542* (Adamidis *et al.*, 1990).

(iii) Crosses like that shown in Fig. 2(a) mapped additional *sab* mutations in some of the *sab* strains listed in Table 1. Before being used in crosses, each strain was purified as described in Methods, and its phenotypic stability was evaluated: no Abs⁻ colonies were found among approximately 200 000 colonies screened by microscopic analysis of plates containing several thousand colonies each.

The *sab* strains were crossed against J650 (an antibiotic-producing strain) and the recombinant progeny were evaluated for the Abs⁻ phenotype. Spores were first selected for the presence of the *strA1* allele. All of these progeny also inherited SCP1_{NF} as well as the J650 9 o'clock region (because the parental plasmid status was NF × UF, as discussed above). Thus, all progeny genomes were recombinant in interval 1. A second selection scheme imposed a requirement for the *his*⁺ allele as well as for *strA1*. With this selection, two Abs⁻ colonies were found among over 100 000 His⁺ Str^r colonies from each of the C542S10 and C542S3 crosses. In contrast, the first selection scheme yielded Abs⁻

colonies at 10-fold higher frequencies. These results suggested the relative order of the *sab* and *absA* alleles drawn in Fig. 2(a): Abs^- colonies could result from crossovers in intervals 1 and 3 in the first selection for Str^r , but would require more crossovers, in intervals 1, 3 and 4, for the $\text{His}^+ \text{Str}^r$ selection.

A *sab* locus maps close to the *absA* locus

As tabulated in Fig. 2(a), crosses with the pseudo-revertant strains all yielded a low percentage of Abs^- progeny. Difficulty in detecting the infrequent *absA1 sab*⁺ recombinants precluded making significant distinctions between the locations of the various *sab* mutations. Because protoplast fusions are potentially capable of increasing the accuracy of recombination analysis due to the higher recombination frequency in such crosses (Hopwood *et al.*, 1977), we explored this procedure. As in $\text{NF} \times \text{UF}$ plasmid-mediated crosses, $\text{SCP1}_{\text{NF}} \times \text{UF}$ protoplast fusions typically result in acquisition of SCP1_{NF} in all progeny (Hopwood *et al.*, 1977). However, in contrast to $\text{NF} \times \text{UF}$ matings, the frequency of unselected parental markers at other loci is not biased toward either parental genotype.

Protoplasts of *sab* strains C542S3 or C542S9 were mixed with J650 protoplasts and recombinant progeny were analysed. Two classes of recombinants were selected: $\text{Str}^r \text{Ura}^+$ and $\text{Str}^r \text{His}^+$. Among these, Abs^- colonies were found at higher frequencies than in plasmid-mediated crosses, as predicted (e.g. 0.6% vs 0.0025% for the *strA1 his*⁺ SCP1_{NF} selection with strain C542S3). Mock protoplast fusions using only the individual *sab* strains, without J650, yielded no Abs^- colonies.

The recombination data for C542S9 suggested the relative order for the *sab* and *absA* alleles shown in Fig. 2(b). This order would be consistent with the higher frequency of Abs^- recombinants found in the $\text{Str}^r \text{Ura}^+$ selection compared to the $\text{Str}^r \text{His}^+$ selection: in the former, two recombination events, in intervals 1 and 4, could produce Abs^- progeny whereas in the latter, three recombination events, in intervals 1 or 2, 4 and 5, would be necessary. The data for C542S3, however, showed no significant difference in recombinant frequencies for the two selections. This observation is discussed further below.

The observed frequencies of recombination between the *sab* and *absA1* alleles did not provide sufficient information to accurately determine their physical distances, and therefore to suggest whether *sab* alleles were intragenic or extragenic. Very little data correlating recombination frequencies with physical distance are available for streptomycetes. To make one such comparison, we measured the recombination frequency between mutations in the *act* gene cluster, which has been completely cloned and sequenced. Using protoplast fusions of strains TK16 and TK18 (Table 1), we obtained 3.7% *act*⁺ recombinants between the *act-141* and *act-117* mutations, which affect the *actIII* and *actIV* loci, respectively, and so the mutations would be approximately 5 kb apart in the *act* cluster (Malpartida &

Strain	Frequency of Abs^- RS100 lysogens
C542S2	2/285
C542S3	3/14
C542S9	24/78
C542S10	3/75

Fig. 3. Mapping *sab* alleles to a 3.2 kb *XhoI*-*XhoI* interval. RS100 carries *absA2* and 5'-truncated *absA1* gene (indicated by 'absA1'). The *absA1*-542 mutation (X) has been mapped to *absA1* (Brian *et al.*, 1996). The *sab* mutation (O) is hypothesized to be in *absA2*. If the *sab* mutant site is covered by the RS100 insert, recombination in interval II, between the two mutant sites, could produce Abs^- lysogens. Shading indicates the expressed copy of *absA*. B, *Bam*HI; X, *Xho*I.

Hopwood, 1986). Considering these data with respect to the *sab-absA1* recombination frequencies suggested that the *sab* mutant sites could lie within the same gene as the *absA1*-542 mutation or very close by.

Isolation of *sab* alleles on specialized transducing phage

The mapping results for the *sab* mutations suggested that they might be close enough to *absA* to lie within the region carried by an 11 kb *absA1*-542-complementing clone, pWC3151, that we had obtained in other experiments (Brian *et al.*, 1996). Accordingly we subcloned DNA from pWC3151 in a temperate phage vector, KC516, for marker rescue experiments. To establish lysogens in *absA1*-542 *sab* strains, we used phage RS100, which carries a 3.2 kb *XhoI* fragment (Fig. 5a) spanning the *absA1* mutant sites (Brian *et al.*, 1996), and also including *absA2*. Fig. 3 illustrates the genotypes expected in such lysogens. In this example, a *sab* mutant site is hypothesized in *absA2* (see below) and three intervals for recombination are delineated. Two considerations are important in interpreting the lysogen phenotypes. First, the RS100 cloned fragment lacks the *absA1* N-

Fig. 4. Crossing the *sab9* mutation onto the phage-cloned insert. RS120 carries the *absA1-542* mutation in the 5'-truncated *absA1* gene. (a) Recombination in intervals I or II could produce Abs⁻ lysogens. (b) Released phage could carry the *sab9* mutation. (c) A phage strain, WC542S9, could transduce C542 to the Pha phenotype. B, *Bam*HI; X, *Xho*I.

terminus and transcription start site; second, *absA1* and *absA2* are predicted to be cotranscribed (T. Anderson, unpublished). Lysogens, therefore, would contain one expressed copy of the *absA* locus and one non-expressed copy; those formed by recombination in interval II would carry the *sab* mutant site in the non-expressed copy and the *absA1-542 sab*⁺ alleles in the expressed copy. Hence, recovery of Abs⁻ lysogens following infection of a *sab* strain by RS100 would indicate that the *sab* mutant site was covered by the cloned *Xho*I fragment. For four strains shown in Fig. 3, recovery of Abs⁻ lysogens following this protocol indicated that the *sab* mutant sites were located within the 3.2 kb *Xho*I interval; for C542S9, the result was confirmed by marker exchange, recovering the *sab* allele on the cloned 3.2 kb *Xho*I fragment.

For marker exchange experiments, the protocol illustrated in Fig. 4 was used. The cloned *Xho*I fragment used to recover the *sab* allele carried the *absA1-542* mutation. This fragment was cloned in phage RS120 (Fig. 5a; Brian *et al.*, 1996) and lysogens were established; Fig. 4(a) shows the genotypes of lysogens established in the Pha strain C542S9. Phage recovered from lysogens (Fig. 4b) were plaque-purified and tested for transduction of the Pha phenotype to C542 (Fig. 4c). As shown, phage WC542S9 was able to transduce the *sab9* allele from C542S9 to C542, creating Pha lysogens. This result confirmed that the *sab9* mutation lay in the 3.2 kb *Xho*I interval and also indicated that *sab9* was sufficient to create the Pha phenotype.

Epistasis relationships of *absA* mutant alleles

The *sab9* mutation's close linkage to *absA1-542* raised the possibility that it might lie within the *absA1* or *absA2* genes. Additional evidence supporting such a

notion came from *absA* mutants' epistasis relationships, which, as discussed below, predicted that certain mutations in *absA1* or *absA2* might suppress the Abs⁻ phenotype of C542.

In previous genetic studies, two kinds of disruption mutations in *absA* caused a Pha *sab*-Type II-like phenotype (Brian *et al.*, 1996; Aceti & Champness, 1998). These mutations (Fig. 5a) were (1) an *ermE* insertion/deletion in strain C420, eliminating much of the *absA1* gene and probably also expression of the downstream *absA2* gene, and (2) an insertional disruption (in strain C430) created by single-crossover integration of a phage-cloned fragment internal to the *absA* transcript (phage RS500 in Fig. 5a). The latter mutation primarily disrupted *absA2* expression; it also truncated the C-terminus of *absA1*, but complementation results suggested that the truncated AbsA1 protein was active *in vivo*. Because both disruptions eliminated *absA2* function, we proposed (Brian *et al.*, 1996) that AbsA2 functions as a negative regulator of antibiotic synthesis.

The *absA* alleles that cause an Abs⁻ phenotype mapped to *absA1*. One hypothesis for their effects is that they lock the *absA* two-component system into a negatively regulating mode. Disruption of *absA2* might thus be epistatic to the *absA1-542* and *absA1-577* mutations. The Pha phenotype of RS500 lysogens established in C542 and C577 (see Methods) confirmed this prediction.

Sequence analysis of Abs⁻ mutant strains

In previous work (Brian *et al.*, 1996), marker rescue experiments were used to localize *absA* mutations responsible for the Abs⁻ phenotype to the *Xho*I–*Bam*HI interval within *absA1* (Fig. 5a). To define the mutant

Fig. 5. (a) Map of the *absA* locus. The *absA1* gene encodes a histidine kinase sensor-transmitter; *absA2* encodes a response regulator. *d7* and *d9* are ORFs which are predicted to be part of the CDA gene cluster (www.sanger.ac.uk/pub/s_coelicolor/sequence). Phage strains RS100, RS120 and RS500 (Table 1) contain the inserts shown; C420 (Table 1) contains the *ermE* replacement shown. (b) Mutational alterations to the AbsA1 and AbsA2 proteins in *absA1* (open triangle) and *sab* (filled triangle) mutant strains. Conserved domains (see text) in two-component systems (Parkinson, 1995; Stock *et al.*, 1995) are indicated. A, *Apal*; B, *Bam*HI; P, *Pst*I; S, *Sac*I; X, *Xho*I.

sites present in strains C542 and C577, the *absA1* gene was sequenced.

The *absA1* mutations found in C542 and C577 are shown in Fig. 5(b). C577 contained a single amino acid change, L253R. C542 contained two amino acid changes (I360L and R365Q). We do not know if both mutations in C542 are required to cause the Abs⁻ phenotype. Sequencing *absA2* in C542 and C577 confirmed that in these strains *absA2* contained no mutations.

The C542 and C577 mutations lie in regions of AbsA1 that are conserved in the histidine kinase sensor-transmitter family: the C542 mutations alter the 'G box', which is involved in nucleotide binding (Stock *et al.*, 1995), and the C577 mutation alters a region, provisionally named the 'X box', and recently proposed to be involved in the aspartylphosphatase activity common to many sensor-transmitters (Hsing *et al.*, 1998).

Sequence analysis of *sab* mutations

To locate *sab* mutations potentially within the *absA* locus, we amplified regions of the *absA1* and *absA2* genes by PCR, and then sequenced the products from two independent amplifications of each mutant strain.

Fig. 5(b) shows the *sab* mutant sites found in this analysis.

absA2 was sequenced in 10 *sab* strains, including those genetically analysed in the experiments discussed above. In C542S9, sequencing revealed an S171W change at the N-terminal end of the predicted helix-turn-helix region.

In one *sab* strain, C577S25, the *absA* locus had undergone a deletion that started within the *absA1* gene and extended through *absA2* into the neighbouring ORF, *d9* (Fig. 5a).

Both C542S9 and C577S25 were Type II *sab* strains with a Pha phenotype. Interestingly, C542S9 was even more strongly pigmented than C577S25 and produced more γ -actinorhodin than any other *sab* strain. This was surprising because if the *absA* system functions as a negative regulator, the phenotype of a Δ *absA1absA2* mutation might be expected to overproduce more strongly than an *absA2* point mutation. Thus this result hints at a complex role for the AbsA2 response regulator in antibiotic production. Another possibility is that deletion of the neighbouring uncharacterized DNA modulates the Pha effect in C577S25.

One Type I strain, C577S20, contained an *absA2* mutation, V29A, that appeared to restore at least some

'normal' AbsA function. It did not alter any of the domains known to be involved in phosphotransfer reactions but affected a region that was expected from the NarL response regulator crystal structure (Baikalov *et al.*, 1996) to be α -helical.

As mentioned above, the strains C577S20 and C577S25 were CDA⁻. For C577S25, this can be explained by the observation that the *sab* mutation is a deletion which extends into a *cda* biosynthetic region (Fig. 5a, b). For C577S20 we do not have an explanation for the CDA⁻ phenotype at this time.

No *absA2* mutations were found in the other strains sequenced. However, the marker rescue experiments discussed above had strongly predicted that the *sab* alleles in the strains tested lay in the 3.2 kb *Xho*I interval (Fig. 3), and it was possible that *absA1* could be mutant in some *sabs*. Therefore, we sequenced *absA1* in two strains: C542S3, a Type I strain that had been used in all of the genetic experiments; and C542S11, a Type II strain used in initial investigations of the pseudo-reversion phenomenon. Both strains proved to contain *sab* mutations in *absA1*, as well as in the *absA1*-542 mutations discussed above.

Strain C542S3 contained the mutation G252V. This amino acid is in the same region of AbsA1 in which the Abs⁻ strain C577 is mutant. In fact, the two mutations affect adjacent residues, posing the questions about whether the *sab3* mutation could suppress the *absA1*-577 mutation and whether the *absA1*-577 mutation could suppress the *absA1*-542 mutation. However, we have not yet tested these possibilities or otherwise addressed whether the various *sab* mutations are allele-specific.

The Type II strain C542S11 contained an *absA1* nonsense mutation in amino acid residue 360. The Pha phenotype may have been due to a polar effect on *absA2* expression. Alternatively, if the nonsense mutation was not polar, the antibiotic might be overproduced because phosphorylation of AbsA2 is necessary for its negatively regulating function, and the mutant lacks AbsA1 kinase activity.

Do *sab* mutations occur outside the *absA* locus?

Two of 10 *sab* strains in which *absA2* was sequenced contained *absA2* mutations and two more strains in which *absA1* was sequenced contained *absA1* mutations. Of the remaining strains, two (C542S2 and C54210) were used in the marker rescue mapping experiments that predicted close linkage of the *absA1*-542 and *sab* alleles. Thus, these would be expected to contain mutations in *absA1*, but could contain mutations in the 2.7 kb uncharacterized region (rightwards of *absA2* in Fig. 5a) cloned in phage RS100 [however, the relatively low recombination frequency of Abs⁻ lysogens (Fig. 3) compared to the *sab9* mutation in *absA2* argues against this possibility]. Altogether, 11 *sab* strains have been used in one of the types of crosses illustrated in Figs 1–3 and in all cases there was evidence for a *sab*

mutation close to *absA*. Nevertheless, the analysis discussed here does not exclude the possibility that *sab* mutations could occur outside *absA*.

Correlation of mapping data with sequencing data and the physical map

The protoplast fusion results with strain C542S9 led us to predict in Fig. 2(b) that the relative order for *absA1*-542 and *sab*, with respect to other genetic markers, would be (in anticlockwise order), *hisA*-*absA1*-*sab*-*uraA*. Considering that *sab9* lies in *absA2*, this would give a relative gene order for *absA1* and *absA2* of *hisA*-*absA1*-*absA2*-*uraA*. We have located the *absA* locus on the ordered cosmid E8 (Redenbach *et al.*, 1996), and the order has been confirmed during the ongoing genomic sequencing (www.sanger.ac.uk/pub/s_coelicolor/sequence).

The protoplast fusion results (Fig. 2b) did not predict a relative order for the *absA1* and *sab3* mutations in strain C542S3, since there was no significant difference in the frequency of *absA1*-542 *sab*⁺ recombinants for the two selections. In light of the close linkage of *absA1*-542 and *sab3* in the *absA1* sequence (Fig. 5b), gene conversion events could have contributed significantly to production of recombinant genotypes, and so distorted apparent recombination frequencies. The marker rescue experiment in Fig. 3 and the plasmid-mediated recombination experiment in Fig. 2 would also be subject to gene conversion effects that could affect ordering of mutations that are close together.

Conclusions

- (i) Non-antibiotic-producing (Abs⁻) mutants of the *absA* locus, which seem to lock the AbsA regulatory system into a negatively regulating mode, contain point mutations in conserved domains of the AbsA1 histidine kinase sensor-transmitter protein.
- (ii) The *absA1* mutants spontaneously acquire suppressive mutations that restore antibiotic synthesis.
- (iii) Plasmid-mediated and protoplast fusion mapping techniques were useful for genetic analysis of suppressive (*sab*) mutations, locating some close to *absA*.
- (iv) Actinophage ϕ C31-derived vectors were useful for marker rescue and marker exchange experiments that verified the existence and location of *sab* mutations and allowed transduction of *sab* mutations from strain to strain.
- (v) Sequence analysis defined *sab* mutant residues in the *absA* two-component system. Some *sab* alleles (Type I) restore apparently normal AbsA function since they restore a wild-type phenotype to Abs⁻ mutants, whereas some (Type II) cause antibiotic overproduction.
- (vi) Antibiotic overproduction in *sab* strains can result from deletion of *absA*, consistent with *absA*'s proposed role as a negative regulator, but the most strongly pigmented *sab* strain contains a point mutation in the

AbsA2 response regulator, suggesting a complex role for the *absA* locus in production of antibiotics.

ACKNOWLEDGEMENTS

We thank Maryuska Calderón for technical assistance and Heather Bobier and Tom Lucier for help with mapping experiments. This work was supported by grants MCB9604055 and MCB9206068 from the National Science Foundation to W.C.

REFERENCES

- Aceti, D. & Champness, W. (1998). Transcriptional regulation of *Streptomyces coelicolor* pathway-specific antibiotic regulators by the *absA* and *absB* loci. *J Bacteriol* **180**, 3100–3106.
- Adamidis, T., Riggle, P. & Champness, W. (1990). Mutations in a new *Streptomyces coelicolor* locus which globally block antibiotic biosynthesis but not sporulation. *J Bacteriol* **172**, 2962–2969.
- Baikalov, I., Schroder, I., Kaczor-Grzeskowiak, M., Grzeskowiak, K., Gunsalus, R. P. & Dickerson, R. E. (1996). Structure of the *Escherichia coli* response regulator NarL. *Biochemistry* **35**, 11053–11061.
- Bibb, M. (1996). The regulation of antibiotic production in *Streptomyces coelicolor* A3(2). *Microbiology* **142**, 1335–1344.
- Brian, P., Riggle, P. J., Santos, R. A. & Champness, W. C. (1996). Global negative regulation of *Streptomyces coelicolor* antibiotic synthesis mediated by an *absA*-encoded putative signal transduction system. *J Bacteriol* **178**, 3221–3231.
- Bystrykh, L. V., Fernández-Moreno, M. A., Herrema, J. K., Malpartida, F., Hopwood, D. A. & Dijkhuizen, L. (1996). Production of actinorhodin-related blue pigments by *Streptomyces coelicolor* A3(2). *J Bacteriol* **178**, 2238–2244.
- Chakraborty, R. & Bibb, M. (1997). The ppGpp synthetase gene (*relA*) of *Streptomyces coelicolor* A3(2) plays a conditional role in antibiotic production and morphological differentiation. *J Bacteriol* **179**, 5854–5861.
- Champness, W. C. (1988). New loci required for *Streptomyces coelicolor* morphological and physiological differentiation. *J Bacteriol* **170**, 1168–1174.
- Champness, W. (1999a). Cloning and analysis of regulatory genes involved in *Streptomyces* secondary metabolite biosynthesis. In *Manual of Industrial Microbiology and Biotechnology*, 2nd edn, pp. 725–739. Edited by A. Demain & J. Davies. Washington, DC: American Society for Microbiology.
- Champness, W. (1999b). Actinomycete phylogeny and development: questions and challenges. In *Prokaryotic Development*. Edited by L. Shimkitts & Y. Brun. Washington, DC: American Society for Microbiology (in press).
- Champness, W. & Chater, K. (1994). Regulation and integration of antibiotic production and morphological differentiation in *Streptomyces* spp. In *Regulation of Bacterial Differentiation*, pp. 61–94. Edited by P. Piggot, C. Moran & P. Youngman. Washington, DC: American Society for Microbiology.
- Chater, K. F. & Bibb, M. J. (1997). Regulation of bacterial antibiotic production. In *Biotechnology*, Vol. 6, *Products of Secondary Metabolism*, pp. 57–105. Edited by H. Kleinkauf & H. von Döhren. Weinheim: VCH.
- Chater, K. F. & Bruton, C. J. (1985). Resistance, regulatory and production genes for the antibiotic methylenomycin are clustered. *EMBO J* **4**, 1893–1897.
- Chong, P. P., Podmore, S. M., Kieser, H. M., Redenbach, M., Turgay, K., Marahiel, M., Hopwood, D. A. & Smith, C. P. (1998). Physical identification of a chromosomal locus encoding biosynthetic genes for the lipopeptide calcium-dependent antibiotic (CDA) of *Streptomyces coelicolor* A3(2). *Microbiology* **144**, 193–199.
- Dyson, P. & Schrempf, H. (1987). Genetic instability and DNA amplification in *Streptomyces lividans* 66. *J Bacteriol* **169**, 4796–4803.
- Fernández-Moreno, M. A., Cabellero, J. L., Hopwood, D. A. & Malpartida, F. (1991). The *act* gene cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *Streptomyces coelicolor*. *Cell* **66**, 769–780.
- Gramajo, H. E., Takano, E. & Bibb, M. J. (1993). Stationary phase production of the antibiotic actinorhodin in *Streptomyces coelicolor* A3(2) is transcriptionally regulated. *Mol Microbiol* **7**, 837–845.
- Guthrie, E., Flaxman, C., White, J., Hodgson, D. A., Bibb, M. J. & Chater, K. F. (1998). A response-regulator-like activator of antibiotic synthesis from *Streptomyces coelicolor* A3(2) with an amino-terminal domain that lacks a phosphorylation pocket. *Microbiology* **144**, 727–738.
- Hodgson, D. & Chater, K. (1981). A chromosomal locus controlling extracellular agarase produced by *Streptomyces coelicolor* A3(2) and its inactivation by chromosomal integration of plasmid SCP1. *J Gen Microbiol* **124**, 339–348.
- Hopwood, D. & Chater, K. F. (1974). *Streptomyces coelicolor*. In *Handbook of Genetics*, vol. 1, pp. 237–255. Edited by R. C. King. New York: Plenum.
- Hopwood, D., Harold, R., Vivian, A. & Ferguson, H. (1969). A new kind of fertility variant in *Streptomyces coelicolor*. *Genetics* **62**, 461–477.
- Hopwood, D., Wright, H., Bibb, M. & Cohen, S. (1977). Genetic recombination through protoplast fusion in *Streptomyces*. *Nature* **268**, 171–174.
- Hopwood, D. A., Bibb, M. J., Chater, K. F. & 7 other authors (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: John Innes Foundation.
- Hsing, W., Russo, F. D., Bernd, K. K. & Silhavy, T. J. (1998). Mutations that alter the kinase and phosphatase activities of the two-component sensor EnvZ. *J Bacteriol* **180**, 4538–4546.
- Kirby, R. & Hopwood, D. (1977). Genetic determination of methylenomycin synthesis by the SCP1 plasmid of *Streptomyces coelicolor* A3(2). *J Gen Microbiol* **98**, 239–252.
- Malpartida, F. & Hopwood, D. A. (1986). Physical and genetic characterization of the gene cluster for the antibiotic actinorhodin in *Streptomyces coelicolor* A3(2). *Mol Gen Genet* **203**, 66–73.
- Narva, K. E. & Feitelson, J. S. (1990). Nucleotide sequence and transcriptional analysis of the *redD* locus of *Streptomyces coelicolor* A3(2). *J Bacteriol* **172**, 326–333.
- Parkinson, J. (1995). Genetic approaches for signaling pathways and proteins. In *Two-component Signal Transduction*, pp. 9–24. Edited by J. A. Hoch & T. J. Silhavy. Washington, DC: American Society for Microbiology.
- Piret, J. M. & Chater, K. F. (1985). Phage-mediated cloning of *bldA*, a region involved in *Streptomyces coelicolor* morphological development, and its analysis by genetic complementation. *J Bacteriol* **163**, 965–972.
- Redenbach, M., Kieser, H. M., Denapate, D., Eichner, A., Cullum, J., Kinashi, H. & Hopwood, D. A. (1996). A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. *Mol Microbiol* **21**, 77–96.
- Rodicio, M. R., Bruton, C. J. & Chater, K. F. (1985). New derivatives

of the *Streptomyces* phage ϕ C31 useful for the cloning and functional analysis of *Streptomyces* DNA. *Gene* **34**, 283–292.

Rudd, B. A. M. & Hopwood, D. A. (1979). Genetics of actinorhodin biosynthesis by *Streptomyces coelicolor* A3(2). *J Gen Microbiol* **114**, 35–43.

Rudd, B. A. M. & Hopwood, D. A. (1980). A pigmented mycelial antibiotic in *Streptomyces coelicolor*: control by a chromosomal gene cluster. *J Gen Microbiol* **119**, 333–340.

Sermonti, G., Petris, A., Micheli, M. R. & Lanfaloni, L. (1977). A factor involved in chloramphenicol resistance in *Streptomyces coelicolor* A3(2): its transfer in the absence of the fertility factor. *J Gen Microbiol* **100**, 347–353.

Stock, J., Surette, M., Levit, M. & Park, P. (1995). Two-component signal transduction systems: structure–function relationships and mechanisms of catalysis. In *Two-component Signal Trans-*

duction, pp. 25–52. Edited by J. Hock & T. Silhavy. Washington, DC: American Society for Microbiology.

Strohl, W. R. (1997). *Biotechnology of Antibiotics*, 2nd edn. New York: Marcel Dekker.

Takano, E., Gramajo, H. C., Strauch, E., Andres, N., White, J. & Bibb, M. J. (1992). Transcriptional regulation of the *redD* transcriptional activator gene accounts for growth-phase-dependent production of the antibiotic undecylprodigiosin in *Streptomyces coelicolor* A3(2). *Mol Microbiol* **6**, 2797–2804.

Wietzorrek, A. & Bibb, M. (1997). A novel family of proteins that regulates antibiotic production in *Streptomyces* appears to contain an OmpR-like DNA-binding fold. *Mol Microbiol* **25**, 1183–1184.

Received 8 March 1999; revised 26 May 1999; accepted 2 June 1999.