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Relationship of an Unstable *argG* Gene to a 5.7-Kilobase Amplifiable DNA Sequence in *Streptomyces lividans* 66

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The relationship between an unstable *argG* gene and a 5.7-kilobase (kb) amplifiable DNA sequence in *Streptomyces lividans* 66 was investigated. Spontaneous, high-frequency Arg mutants deleted for this gene typically contain 200 to 300 copies of the tandemly reiterated sequence. A library of *S. lividans* 66 (strain 1326) wild-type genomic DNA was prepared in the vector λ Charon 35. Chromosome walking over 44 kb established that *argG* is located 25 kb distant from a duplicated amplifiable DNA structure. A sequence was characterized, located farther distal from the amplifiable structure, containing strong homology with an internal sequence of the amplifiable DNA, which may have a role in the deletion of *argG*. Genetic mapping showed that *argG* and the 5.7-kb amplifiable sequence are linked to another unstable gene, determining chloramphenicol resistance (Cam^r) and that together these genes may be located in a silent chromosomal arc.

Many different streptomycete species exhibit genetic instability whereby certain phenotypes are lost spontaneously at frequencies between 10^{-3} and 10^{-2} (reviewed in references 16 and 34). In the examples for which genes encoding unstable phenotypes have been cloned, further analysis of mutant genomes demonstrated that the high-frequency mutations arose by deletion of the relevant gene (11, 18, 26, 33, 39). Amplification of particular DNA sequences has often been observed to accompany the loss of unstable genes (1, 8, 11, 33). *Streptomyces lividans* 66, for example, exhibits instability of chloramphenicol resistance (Cam^r) and arginine biosynthesis; the latter mutation is associated with amplification of a 5.7-kilobase (kb) DNA element (1, 2), or amplified DNA sequence (ADS [8, 9]).

Similar high-frequency mutations in arginine biosynthesis have been found in variant strains of several other *Streptomyces* spp. (19, 26, 27, 30, 36), although they are not correlated with amplification of DNA sequences. For *S. cattleya*, the gene for argininosuccinate synthase, *argG*, has been cloned and used to demonstrate that the Arg phenotype in variant strains results from deletion of this gene (26, 39). Likewise, a gene complementing either a regulatory or a structural defect of nitrosoguanidine-induced *argG* mutants of *S. coelicolor* A3(2) and *S. lividans* 66 has been cloned and shown to be deleted in the mutants (20), although, in contrast to the *S. lividans* Arg mutants isolated by Altenbuchner and Cullum (1), ADS were not detected in the relevant mutant genomes.

With the wide possibilities of *S. lividans* for genetic studies, this organism provides an attractive system for further investigation of both unstable genes and related DNA amplifications. In this work we confirm genetic data that the spontaneous, high-frequency mutation found in *S. lividans* 66 Arg$^-$ variants, which correlates with the amplification of the 5.7-kb ADS in mutant genomes, results from the deletion of the structural gene for argininosuccinate synthase. We also demonstrate the relationship of the *argG* gene to the duplicated 6.8-kb amplifiable unit of DNA (AUD; 8, 9) in the wild-type chromosome, from which the amplification arises. Furthermore, we have identified dispersed regions of DNA homology which may have a role in deletion formation.

S. lividans exhibits a stepwise pathway of instability of Cam^r and *argG* (1, 7). Both these spontaneous high-frequency mutations were previously identified in *S. coelicolor* A3(2) (5, 10, 36), but it was not possible to locate these genes to fixed positions on a genetic map (10, 35, 36). Genetic transposition was offered as an explanation, but, subsequently it has been argued that some of the original data were incorrectly interpreted (4). In this paper we present data unambiguously mapping these genes to a specific arc of the *S. lividans* chromosome. Although transposition of these genes is not formally excluded by our data, we believe that the experimental design may have given rise to the earlier reported ambiguities.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Bacterial strains used in this study are listed in Table 1. *S. lividans* M7-1 (*pro-2 spc-1*) containing the conjugative plasmid pIJ303 (22) was derived from a cross between strains 3078(pIJ303) (*his-2 leu-2 spc-1*) and TK64 (*pro-2 str-6*), both gifts from the John Innes Institute, Norwich, England (17). Strain M417 (*his-2 leu-2 ura-6 ath-8 str-6 argG* Cam^s) was a spontaneous variant isolated from John Innes strain 3198 (17). Antibiotic concentrations used and culture conditions for streptomycete strains are described in the accompanying paper (7). Crosses were made on plates containing complete medium (7), and recombinants were recovered on suitably supplemented minimal media (15).

Standard culture conditions for *Escherichia coli* strains were used (25). All transformations were into F$^-$ Z$^-$ ΔM15 (32) unless otherwise stated. Genetic complementation of the *argG* mutation of W4183 by pSL100 and pSL101 was tested by growth on Davis-Mingioli salts minimal medium (6) with or without the addition of 40 μg of arginine per ml.

Purification and in vitro manipulation of DNA. Total DNA was isolated from *Streptomyces* strains by a method favoring purification of high-molecular-weight DNA (15). Plasmid DNA was prepared from *E. coli* by the method of alkaline lysis (3). Bacteriophage DNA was isolated by standard

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TABLE 1. Bacterial strains

Strain	Relevant phenotype or genotype (plasmid status)	Source (reference)
S. lividans 66		
1326	Wild type (SLP2, SLP3)	John Innes Institute (17)
TK64	*pro-2 str-6*	John Innes Institute (17)
3078	*his-2 leu-2 spc-1* (pIJ303)	John Innes Institute (17)
3198	*his-2 leu-2 ura-6 ath-8 str-6*	John Innes Institute (17)
M7-1	*pro-2 spc-1* (pIJ303)	This paper
M417	Cam^s *his-2 leu-2 ura-6 ath-8 str-6 ΔargG*	This paper
E20	Cam^s *ΔargG* (ND)[a]	Accompanying paper (7)
E. coli		
F$^-$ Z$^-$ ΔM15 *recA*	(*lac pro*) *thi* φ80 d*lacZ* ΔM15 *ara rpsL recA*	B. Mueller-Hill (32)
K802 *recA*	RglA RglB (McrA McrB) *hsdR2 galK2 galT22 supE44 lacY1 metB1*	F. Blattner
W4183	*argG78 rpsL257*	B. Bachmann (14)

[a] ND, Not determined.

procedures (25). Digestion of DNA with restriction endonucleases and gel electrophoresis were carried out by standard techniques.

Construction of an *S. lividans* 66 genomic DNA library. A library of *S. lividans* 66 genomic DNA was prepared in λ Charon 35 (24) with a *recA* K802 strain as bacterial host. Genomic DNA from the wild type, strain 1326, was partially digested with *Mbo*I and size fractionated by sedimentation on sucrose gradients (25). λ Charon 35 DNA was cleaved with *Bam*HI, and vector arms were resolved after sedimentation on sucrose gradients. Left and right vector arms and the *Mbo*I partially digested genomic DNA between 12 and 20 kb in size were ligated with T4 DNA ligase (Boehringer GmbH, Mannheim, Federal Republic of Germany). Ligation mixtures were packaged in vitro and plaque amplified by standard techniques (25). A DNA insert containing the *S. lividans* 66 *argG* gene was cloned by isolating *Bam*HI-cleaved genomic DNA in the size range 13 to 14 kb by electroelution from a preparative agarose gel (25). This DNA was ligated with *Bam*HI-digested λ Charon 35 by using T4 DNA ligase, and the mixture was packaged as before.

Construction of recombinant plasmids. To clone parts of the 5.7-kb ADS found in *argG* variants of *S. lividans* 66, DNA from variant strain E20 (7) was cleaved with *Sal*I, and amplified restriction fragments were isolated from an agarose gel by electroelution. These DNA fragments were ligated with *Sal*I-cleaved pUC8 (41) by using T4 DNA ligase. The recombinant plasmid structures were confirmed by double digestion of plasmid DNA with *Pst*I, *Pvu*II, *Sst*II,and *Bam*HI, each in combination with *Sal*I, and comparing the resulting restriction fragments with the amplified bands present in similarly cut genomic DNA from strain E20. One recombinant plasmid obtained this way contained the amplified 1.1-kb *Sal*I fragment; this fragment was further checked by hybridizing back the fragment purified from the plasmid against *Pst*I-digested genomic DNA of strains E20 and the wild type, 1326.

To subclone the presumptive *argG* gene of *S. lividans* 66 and test its ability to function in *E. coli*, a 2.2-kb partially digested *Bgl*II DNA fragment from phage SL41 was isolated by electroelution and ligated with *Bam*HI-digested pUC8. The structures of the recombinant plasmids were confirmed by restriction with endonucleases *Bgl*II, *Eco*RI, and *Hin*dIII, hybridization with an *S. cattleya argG* probe, and hybridization of the plasmid insert back against *S. lividans* wild-type DNA. pSL100 and pSL101, containing the 2.2-kb insert in opposite orientations, were used to transform W4183.

pSL110 was constructed by ligation of a purified 2.3-kb *Bam*HI-*Bgl*II DNA fragment from SL41 with *Bam*HI-cleaved pUC8.

DNA hybridization analysis. DNA was transferred to nitrocellulose filters by the method of Southern (38). Preparation of biotinylated DNA hybridization probes, hybridization, and visualization of hybridized probes were done by established methods (23, 31, 37), described more extensively in the accompanying paper (7). Plaque lifts onto nitrocellulose filters were done by standard techniques (25), except that after baking, to eliminate biotin derived from lysed *E. coli*, we incubated the filters for 30 to 60 min at 37°C with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.5 mg of proteinase K per ml and 0.1% sodium dodecyl sulfate. Filters were subsequently hybridized with biotinylated probes prior to detection.

RESULTS

Hybridization with a cloned *argG* gene from *S. cattleya* as DNA probe. The plasmid pMA4 is a pBR322-based vector containing a 5.5-kb *Bam*HI restriction fragment derived from *S. cattleya* and can complement an *argG* mutant of *E. coli* (26). This plasmid was used as a DNA hybridization probe to test genomic DNAs isolated from wild-type *S. lividans* and both Arg$^+$ and Arg$^-$ variants, the majority of the latter containing the 5.7-kb ADS. The probe hybridized strongly to a 13.5-kb *Bam*HI restriction fragment from wild-type and Arg$^+$ variants, but did not hybridize detectably to any of the DNAs tested from Arg$^-$ variants (data not shown). It was concluded that significant homology exists between *S. cattleya* and *S. lividans* 66 *argG* genes and that this sequence was deleted in Arg$^-$ variants of *S. lividans* 66. The *S. lividans* 66 *argG* gene, contained in pSL101, was subsequently hybridized against genomic DNA of the wild type and variants. No signal could be detected in DNAs from Arg$^-$ mutants (data not shown).

Genomic mapping of the *argG* gene and the duplicated 6.8-kb AUD. The plasmid pJD201, containing the 1.1-kb *Sal*I restriction fragment of the 5.7-kb ADS, was used as a DNA hybridization probe against an *S. lividans* 66 genomic library of 3,000 Charon 35 recombinant bacteriophages. Three phages containing homology to the probe were isolated: SL32, SL33, and SL35. SL32 and SL35 contained the

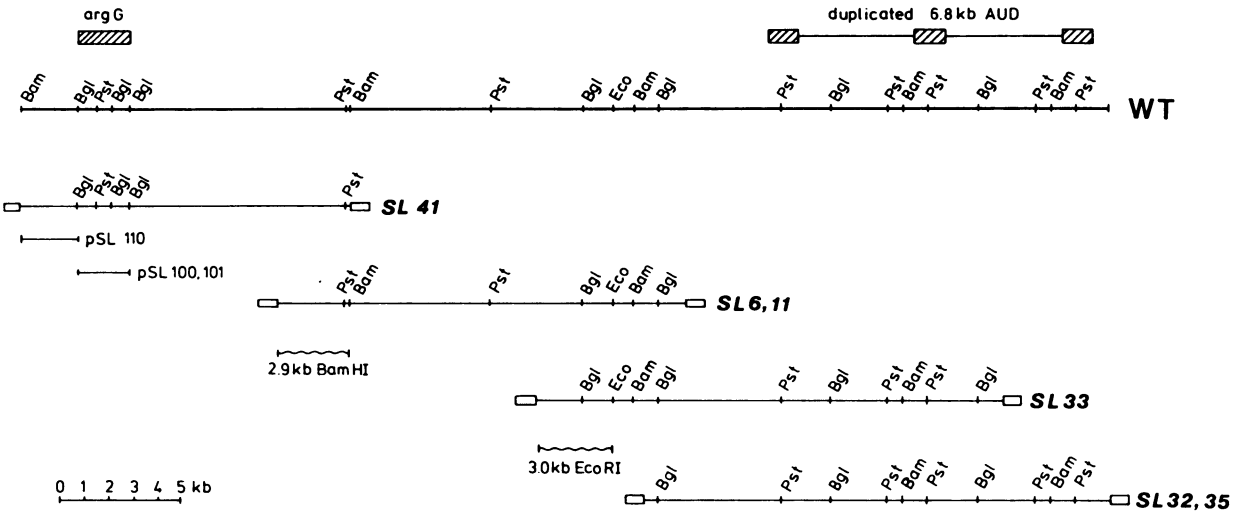

FIG. 1. Restriction map of the 44-kb *argG*-6.8-kb AUD chromosomal region. Locations of *argG* and the amplifiable structure are indicated above the main map, and the representative phages containing the region are shown below. The DNA fragments containing *argG* and its left-hand flanking sequence, subcloned in pSL100/1 and pSL110, respectively, were derived from phage SL41 as indicated. The 2.9-kb *Bam*HI and 3.0-kb *Eco*RI hybridization probes (〰) were derived from phages SL11 and SL33, respectively. Abbreviations: Bam, *Bam*HI; Bgl, *Bgl*II; Eco, *Eco*RI; Pst, *Pst*I.

complete duplicated 6.8-kb AUD structure, analyzed in the accompanying paper (7) and equivalent to that of strain TK64 (2). SL33 included a part of the AUD and sequences from the left-hand flanking region (Fig. 1).

pMA4, containing the *S. cattleya argG* gene, was also used as a probe to isolate two recombinant phages containing homology: SL6 and SL11. Restriction endonuclease analysis of both sets of recombinant phages with *Sma*I, *Ava*I, or *Sst*II revealed common DNA restriction fragments in phages SL6 and SL11 which were also present in phage SL33. A 3-kb *Eco*RI fragment, containing the putative region of overlap between the phages, was isolated by electroelution from *Eco*RI-digested SL33 DNA and used as a DNA hybridization probe. Hybridization of this probe to identical *Sma*I restriction fragments of SL6, SL11, and SL33 confirmed the region of overlap contained within these phages (Fig. 1).

None of the recombinant phages containing homology with the pMA4 probe included the 1.5- and 0.7-kb *Bgl*II restriction fragments previously found in a region suggested to encode the *S. lividans argG* gene (20). This anomaly could be rationalized if this previously reported *argG* clone in fact encoded a regulatory gene, not a homologous structural gene, or if the representative phages we isolated contained homologous flanking sequences to the structural gene of *S. cattleya*, but not the gene itself. To distinguish between these possibilities, and as an alternative strategy, a fraction of *Bam*HI-digested wild-type DNA in the size range of 13 to 14 kb, containing the band that hybridized with pMA4, was cloned in λ Charon 35. A recombinant phage, SL41, isolated by plaque hybridization with the pMA4 probe, contained both the 1.5- and 0.7-kb *Bgl*II restriction fragments. This result implicated the second of the above explanations: SL6 and SL11 contained a flanking sequence homologous to *S. cattleya argG*, and SL41 contained the structural gene itself. Comparison of phages SL41, SL6, and SL11 by restriction analysis revealed identical-sized *Sau*3A restriction fragments present in all three phage inserts. The region of overlap was confirmed by hybridization of a 2.9-kb *Bam*HI restriction fragment probe derived from SL11 to the identical *Sau*3A fragments from all three phages. This enabled us to map the entire *S. lividans* 66 chromosomal region containing *argG* and the duplicated 6.8-kb AUD (Fig. 1). It was noted that the putative *argG* structural gene of *S. lividans* 66, located in SL41, and a sequence flanking the same gene of *S. cattleya*, homologous with a sequence in phages SL6 and SL11, are not colinear in the *S. lividans* 66 genome.

It was previously found that several different sequences derived from amplified regions of genomes of streptomycete variants cannot be stably propagated in recombinant plasmids in *E. coli* hosts (H. Schrempf, P. Dyson, and P. Groitl, unpublished observations). The λ Charon 35 vector has been engineered to eliminate the phage Red function while retaining *gam*, whose product inhibits exonuclease V of *E. coli* (24). Recombinant clones can therefore be propagated in *recA* mutant bacteria, whose phenotype is also then effectively RecBC$^-$, providing a double block to recombination and potentially enhancing the stability of inserted DNA. The host, K802 *recA*, is RglA and RglB (McrA and McrB), mutations that allow establishment and propagation of cytosine-methylated DNA in *E. coli* (28, 29), which could be important for the cloning of G+C-rich streptomycete DNA. To discount any possibility of rearrangements occurring in originally isolated phages spanning the 44-kb chromosomal region in question, restriction fragments derived from each recombinant phage insert were hybridized back against different restriction digests of the wild-type genomic DNA. For each probe the hybridization patterns obtained were in complete accord with the genomic map constructed from analysis of the phage library.

Since the *S. cattleya argG* gene was isolated by complementation of an *argG* mutation in *E. coli* (26), we tested the ability of the presumptive *S. lividans* 66 *argG* gene to function in *E. coli*. A partial *Bgl*II restriction fragment containing both the 0.7- and 1.5-kb fragments was subcloned from SL41 into the *Bam*HI site of pUC8. The resulting constructs, pSL100 and pSL101, containing the 2.2-kb fragment in both orientations, were introduced into *E. coli* W4183, and both were shown to complement the *argG* mutation of that strain.

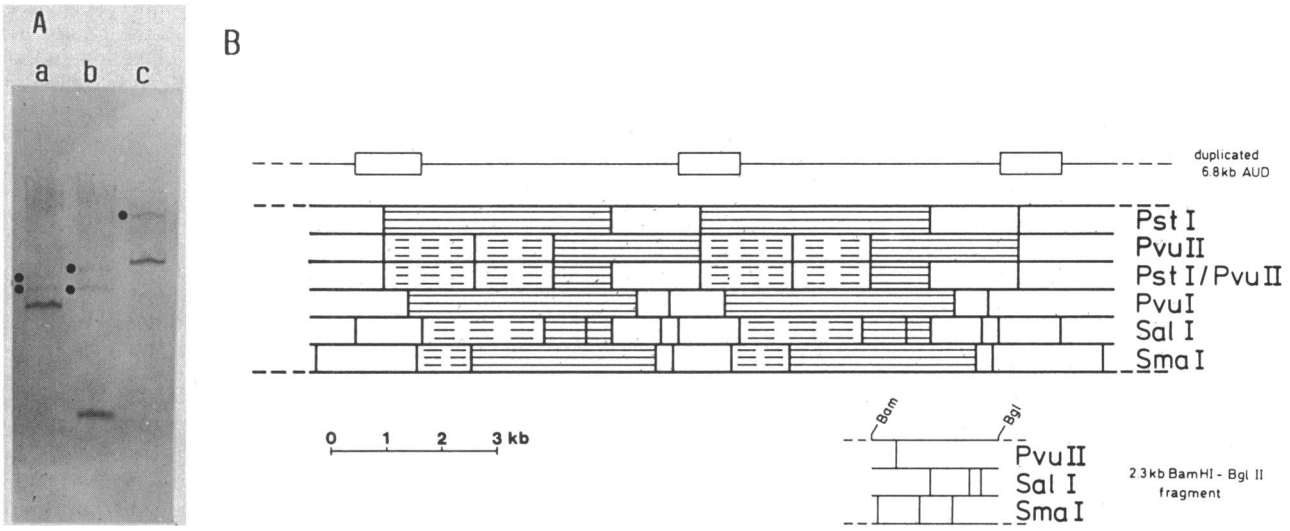

FIG. 2. Homology between the 2.3-kb *Bam*HI-*Bgl*II fragment and the AUD. (A) Hybridization of the *argG* flanking sequence isolated from pSL110 against wild-type *S. lividans* 66 chromosomal DNA cleaved with *Sac*I (lane a), *Bgl*II (lane b), or *Bcl*I (lane c) reveals strong hybridization against 5.5-, 2.5-, and 8.3-kb bands, respectively, each containing this sequence, and weaker hybridization against AUD-containing fragments, indicated by dots. Lanes: a, 5.7 and 6.0 kb; b, 5.7 and 6.6 kb; c, 15 kb. (B) The same probe, hybridized against different SL35 DNA restriction digests, defines the regions of homology present in the duplicated 6.8-kb AUD structure. Strong homology is denoted by unbroken shading of relevant restriction fragments, and weaker homology is shown by broken-line shading. For comparison, a restriction map for the same enzymes of the 2.3-kb region derived from the left-hand flanking sequence of *argG* is shown below. *Pst*I and *Pvu*I do not cut within this sequence.

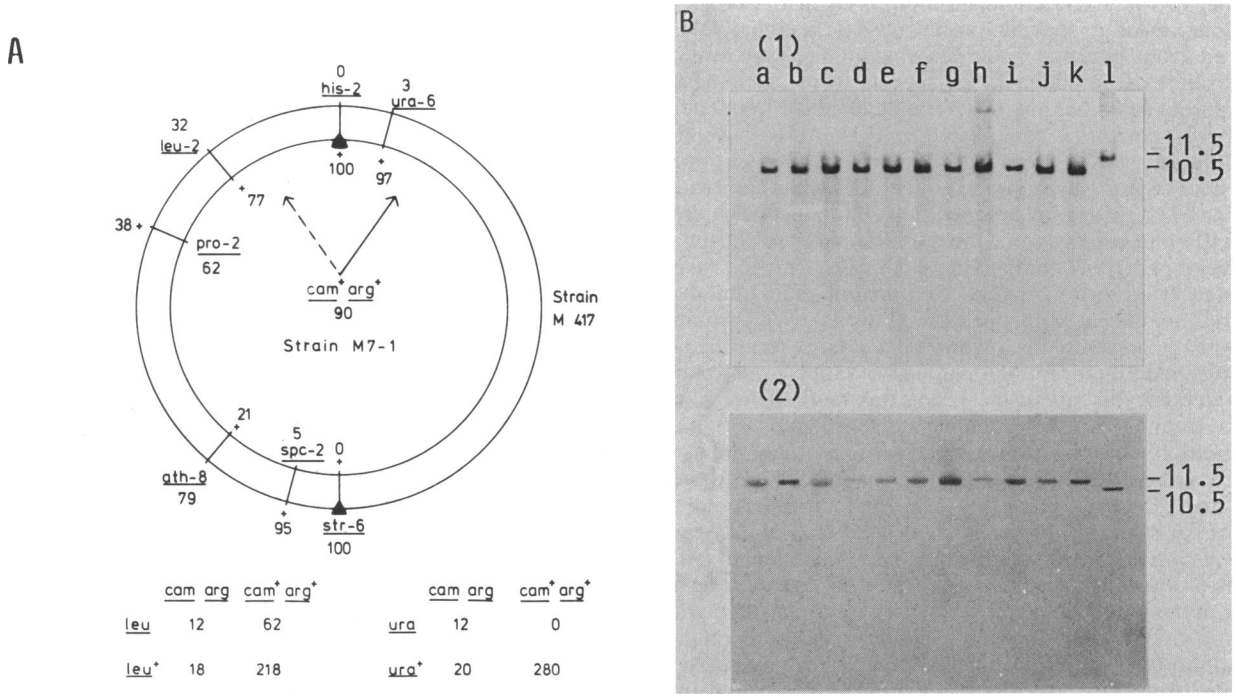

FIG. 3. Genetic mapping of *argG*, *cam*, and the 6.8-kb AUD. (A) Strains M7-1 (inner circle) and M417 (outer circle) were crossed, and *his-2*$^+$ *str-6* recombinants were selected (▲, selection points). Numbers around the circles are frequencies of alleles among 311 recombinants scored. Segregation of *cam* and *arg* alleles with respect to the *leu* and *ura* alleles is indicated below the maps. Abbreviation: Cml^r, chloramphenicol resistance phenotype. (B) The AUD proximal deletion endpoint of M417 was monitored in the cross by hybridization of the 3.0-kb *Eco*RI probe against *Bam*HI-cleaved chromosomal DNA from recombinants. The probe hybridizes against an 11.5-kb *Bam*HI fragment of the M7-1 parent and a 10.5-kb fragment of M417. (Blot 1) Cam^s *arg* recombinants (lanes a to j), M417 (lane k), and M7-1 (lane l). (Blot 2) Cam^r *arg*$^+$ recombinants (lane a to j), M7-1 (lane k), and M417 (lane l). Sizes of hybridizing bands are indicated in kilobases.

DNA homology between a sequence flanking *argG* and the AUD. To extend the genomic map to the left of the *argG* gene, a 2.3-kb *Bam*HI-*Bgl*II fragment flanking the *argG* gene and subcloned in pSL110 was labeled and hybridized against the λ Charon 35 gene bank. Analysis of several recombinant phages so isolated showed that they were identical to previously isolated phages containing the 6.8-kb AUD region. The region of homology was further investigated by hybridizing the same probe against different restriction digests of DNA of the AUD-containing phage SL35 (Fig. 2B). Stronger homology with certain restriction fragments and weaker homology with others was detected, defining the total area of homology to an area of about 4 kb. To exclude the possibility that such a result could arise from either an artifact of cloning or inadvertent examination of clones derived from an original circular DNA species, the probe was hybridized back against wild-type chromosomal DNA. Under stringent conditions the probe hybridized strongly against chromosomal fragments containing the 2.3-kb *Bam*HI-*Bgl*II sequence and less strongly against the relevant fragments derived from the 6.8-kb AUD region (Fig. 2A). The region was mapped with restriction enzymes to compare it with the homologous AUD sequence (Fig. 2B). We could find no similarity between the restriction maps of the two sequences.

Genetic mapping of Cam^r, *argG*, and the 6.8-kb AUD region. Figure 3A illustrates the mapping of Cam^r and *argG*. The parental *argG* Cam^s strain, M417, was judged suitable for this analysis as a result of previous investigation of the DNA deletion responsible for its *argG* genotype and single-copy 6.8-kb AUD status (7). The defined deletion endpoint proximal to the AUD could be monitored in the cross by hybridization of a specific probe against genomic DNAs prepared from parental and recombinant progeny strains. The absence of amplified DNA species in the genomes of either parent ruled out any interference on allele frequencies that these sequences can introduce (11). M417 was at a considerable growth disadvantage in mixed lawns with M7-1, necessitating appropriate adjustment of parental ratios (M417 to M7-1, 250:1) to generate equal numbers of parental types after harvesting. Since the parental M417 strain sporulated very poorly in comparison with M7-1, mycelia were harvested from plate crosses, fragmented, and plated on selective media at appropriate dilutions. Well-isolated recombinant single colonies were subsequently repurified on selective media before being patched on appropriate plates to test genotype frequencies. Using this method, we found that the frequency of heteroclones generated was 3%.

cam exhibited complete linkage with the *argG* locus in crosses, and consideration of allele frequencies placed them either between *his-2* and *leu-2* in the left-hand arc, or between *str-6* and *ura-6* in the right-hand arc. Since segregation of *cam* and *argG* was essentially independent of *leu-2*, but not of *ura-6*, the latter position was indicated. A deviation from the published allele frequency for the *ura-6* marker (17) was noted and rationalized in terms of the relative fitness (shorter generation times and larger colony sizes) of the Cam^r Arg$^+$ Ura$^+$ recombinant progeny recovered over their Cam^s Arg$^-$ Ura$^-$ counterparts. Taking into account this bias favoring the recovery of Ura$^+$ recombinants, the Cam^r determinant, *argG*, and the 6.8-kb AUD can be located clockwise from 17 min, the published position of *ura-6*.

The fidelity of the deletion responsible for the Arg phenotype of M417 and the 6.8-kb AUD status in recombinant progeny of the cross was tested. Chromosomal DNA was isolated from 10 Cam^s Arg$^-$ Ura$^+$/Ura$^-$ transconjugants and 10 Cam^r Arg$^+$ Ura$^+$ transconjugants. The 3-kb *Eco*RI DNA probe (see above), which defines the AUD proximal deletion endpoint within the *argG*-AUD intervening sequence of M417 (7), was used to probe these DNAs. All Cam^s Arg$^-$ transconjugants tested inherited the same deletion endpoint, and all Cam^r Arg$^+$ transconjugants examined had the parental M7-1 arrangement (Fig. 3B). All recombinants tested contained the single 6.8-kb AUD copy present in both parents (data not shown).

DISCUSSION

Amplification of a 5.7-kb ADS is found in variants derived from *S. lividans* 66 that have undergone stepwise mutation to Cam^s Arg (1, 7). To investigate the relationship of unstable genes and the amplified DNA sequence in the wild type, we analyzed a gene bank of *S. lividans* 1326 DNA constructed in a λ Charon 35 cloning vector. The isolation and characterization of representative phages allowed us to construct a colinear map of a 44-kb region of the *S. lividans* 66 genome containing both *argG* and the duplicated 6.8-kb AUD from which the 5.7-kb ADS is generated (Fig. 1). To our knowledge, this is the first example in which an unstable *Streptomyces* gene has been specifically located in relation to a DNA element which is amplified in mutant strains in which the gene is deleted.

A 2.2-kb region implicated to contain the *S. lividans argG* gene (20) was confirmed to contain the structural *argG* gene by subcloning of this sequence in an *E. coli* vector and subsequent complementation of an *E. coli argG* mutant. Complementation occurred irrespective of the orientation of the cloned gene, suggesting that the gene promoter is recognized by *E. coli* RNA polymerase, as indicated for the *S. cattleya argG* gene (26) and several other streptomycete promoters (21).

The extent of deletions in Cam^s Arg mutants of *S. lividans* 66 is at least 25 kb and is possibly greater (7). Analysis of the sequence immediately to the left of and flanking the *argG* gene showed it to contain significant homology with an internal sequence of the 6.8-kb AUD. Dispersed homologous DNA sequences such as these may play a significant role in the generation of deletions, in a manner similar to the role that dispersed copies of homologous rDNA genes in the *E. coli* chromosome can play in providing substrates for recombination (12, 13). The pattern of spontaneous segregation of *S. lividans* is stepwise: mutation to Cam^s followed by deletion of *argG* and coupled DNA amplification. We speculate that a series of deletions occurs in the specific chromosomal arc in question, the last of which is the loss of *argG*. The region immediately leftward of *argG* may provide homology for both the penultimate and last steps in deletion formation. It has been previously suggested that repetitive DNA sequences, which are found as 4 to 10% of total DNA in streptomycete species, may provide hot spots for recombination events (40).

Previous attempts to genetically map the Cam^r determinant and *argG* genes in *S. coelicolor* A3(2) revealed ambiguities (10, 35, 36). We believed that the presence in the genome of one parental type of large DNA deletions and amplified DNA sequences could drastically affect segregation patterns, giving rise to ill-defined map positions. To address this question for *S. lividans*, we used a well-characterized Cam^s Arg parental strain, lacking amplified DNA sequences, with a defined deletion endpoint proximal to the 6.8-kb AUD and not grossly affected in morphological traits (perhaps suggesting that a small deletion had occurred

in this strain). Our analysis provided an unambiguous map region for the Cam^r determinant, *argG*, and the 6.8-kb AUD. Given the paucity of markers in the right-hand chromosomal arc in question, a precise position cannot yet be ascribed. However, linkage to the *ura-6* marker, located at 2 o'clock on the *S. lividans* chromosome (7), invites speculation that these genes reside in the so-called silent chromosomal arc, around 3 o'clock. We are investigating this possibility further. The fact that *S. lividans* can tolerate the deletion of large genomic regions may be due to their occurring in relatively nonessential parts of the genome.

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