The *Streptomyces coelicolor* A3(2) *bldB* region contains at least two genes involved in morphological development

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Streptomyces coelicolor A3(2) *bldB* mutants are blocked in the formation of aerial hyphae. A phage library of wild-type *S. coelicolor* DNA was used to isolate recombinant phages which restore wild-type morphological development to several *bldB* mutants. Of several mutations, one, *bld-28*, previously mapped at *bldB* was not complemented by the cloned region, indicating that the *bldB* locus is composed of at least two distinct genes. Partial localization of *bldB*-complementing activity showed that a 1.5 kb fragment is sufficient for complementation of the *bld-15* mutation whereas *bld-17* requires the same region as well as additional sequences. Under stringent conditions, genomic DNA hybridizing to the cloned sequences was absent from other *Streptomyces* species, including the closely related *Streptomyces lividans* 66. DNA sequences causing marked plasmid structural instability in *S. coelicolor*, but not in *S. lividans*, are also located in this region.

Introduction

Colony development in *Streptomyces coelicolor* A3(2) has been the subject of a number of genetic studies, both traditional and molecular in approach (for a review see Chater, 1984). On solid medium, a vegetative substrate mycelium is formed during the first few days of growth, which then sends up aerial branches above the colony surface, giving it a hairy, white macroscopic appearance. The aerial hyphae develop long chains of haploid spores, held together by a delicate fibrous sheath. These provide the organism with a means of dispersal. Colony differentiation is accompanied by the synthesis of a wide variety of secondary metabolites, not required for growth. In *S. coelicolor* these include four antibiotics, two of which (actinorhodin and undecylprodigiosin) are easily recognized because they are pigmented. The regulatory mechanisms controlling morphological development in this system present an attractive subject for the study of the temporal and spatial control of gene expression.

One approach to studying morphological development is through the use of morphological mutants. In this paper, we are concerned with *bld* (bald) mutants which make vegetative mycelium but do not form aerial hyphae. At least seven classes of *S. coelicolor* *bld* mutants have been defined by phenotypic and genetic analysis:

bldA, *bldB*, *bldC*, *bldD* (Hopwood, 1967; Merrick, 1976), *bldF* (Puglia *et al.*, 1986; A. M. Puglia, personal communication), *bldG* and *bldH* (Champness, 1988). The *bld* genes map at a number of locations around the *S. coelicolor* circular linkage map. The morphological deficiencies of *bldA*, *bldD*, *bldG* and *bldH* mutants can be phenotypically corrected by changing the carbon source (Merrick, 1976; Champness, 1988). This suggests that there are alternative pathways for aerial mycelium formation in this species.

bldB mutants form very smooth colonies, leathery in texture, apparently composed only of substrate mycelium. Like most *bld* mutants, they also have defects in antibiotic production. Eight independent *bld* mutants have been assigned to *bldB*, which maps in the 5 o'clock region of the *S. coelicolor* linkage map (Merrick, 1976; Champness, 1988). Here we describe the isolation of a DNA fragment from the wild-type *bldB* region and the finding that the region is complex in organization.

Methods

Bacterial strains. The *Streptomyces coelicolor* A3(2) strains used in this work are listed in Table 1. *Streptomyces parvulus* ETH 2283, *Streptomyces glaucescens* ETH 22794, *Streptomyces scabies* ETH 3171, *Streptomyces peucetius* ETH 3180, *Streptomyces noursei* ETH 3027, *Bacillus thuringiensis* var. *israeliensis* and *Amycolatopsis mediterranei*

Table 1. *Streptomyces coelicolor** and ϕ C31 strains used in this work

Strain*	Genotype	Reference
Bacteria†		
M145	Prototroph	Hopwood <i>et al.</i> (1985)
J1501	<i>hisA1 uraA1 strA1 pgl</i>	
J650	<i>cysD18 mthB2 agaA7</i>	Merrick (1976)
J668	<i>cysD18 mthB2 agaA7 bldA39</i>	
J669	<i>cysD18 mthB2 agaA7 bldB43</i>	M. J. Merrick (unpublished)
J701	<i>mthB2 agaA7 bld-15</i>	
J703	<i>mthB2 agaA7 bld-28</i>	
J704	<i>mthB2 agaA7 bld-17</i>	
J95	<i>cysD18 strA1 whiH119</i>	This paper
J118	<i>cysD18 strA1 whiG71</i>	
P101	<i>bld-15</i> derivative of J1501	
Phage‡		
φC31 KC301	<i>c⁺ attP⁺</i> , contains <i>tsr</i>	Hopwood <i>et al.</i> (1985)
φC31 KC401	Δ <i>c</i> Δ <i>attP</i> , contains <i>vph</i>	
φC31 KC625/626§	KC401 carrying <i>bldB⁺</i> insert	This paper
φC31 KC628	<i>c⁺</i> derivative of KC626	
φC31 KC628- <i>bld-15</i>	KC628 carrying <i>bld-15</i>	
φC31 KC628- <i>bld-17</i>	KC628 carrying <i>bld-17</i>	

* All strains were originally derived from *S. coelicolor* A3(2).

† For marker designations see Merrick (1976). Plasmid status is not given.

‡ *c*, repressor gene; *attP*, phage attachment site; Δ , deletion; *tsr*, thiostrepton resistance gene; *vph*, viomycin resistance gene.

§ Two orientations of the cloned insert.

(formerly *Nocardia*) ATCC 13685 were from the Microbiology Institute, Swiss Federal Institute of Technology, Zürich, Switzerland. *Streptomyces lividans* 66 (John Innes strain 1326; Lomovskaya *et al.* 1972), *Escherichia coli* ED8767 (Murray *et al.*, 1977) and *S. coelicolor* A3(2) strains were from the John Innes Institute collection.

Phage and plasmid vectors. Derivatives of the temperate bacteriophage ϕ C31 (Lomovskaya *et al.*, 1972) were used and are listed in Table 1. Conditions for phage propagation, enumeration and crosses were as described by Hopwood *et al.* (1985). ϕ C31 KC628 was obtained from a cross between strains KC626 and KC301 by screening for phages released from a double lysogen which formed turbid plaques and transduced viomycin resistance. The *Streptomyces* plasmid vectors pIJ702 (Katz *et al.*, 1983), pMEA3 (Hintermann *et al.*, 1985), pIJ364 (Kieser *et al.*, 1982) and pIJ486/7 (Ward *et al.*, 1986) are derivatives of the multicopy plasmid pIJ101 (Kieser *et al.*, 1982).

Bacterial growth. YEME broth (Hopwood *et al.*, 1985) was the culture medium for the preparation of *Streptomyces* mycelial fragments. Following harvest by centrifugation, pellets were washed in sterile distilled water and stored in 20% (v/v) glycerol at -20°C . R2YE agar (Hopwood *et al.*, 1985) was used for routine *Streptomyces* cultures, for the preparation of spores, in the shotgun cloning experiment and for the assessment of the sporulation status of *bld* mutants. Minimal medium agar (MM; Hopwood *et al.*, 1985) containing mannitol (0.5%, w/v) as carbon source was used to assess the sporulation status of *whi* mutant strains. For light microscopy, coverslip impression preparations were made by the method of Chater (1972).

DNA manipulations. Plasmid and phage DNA were prepared as described by Hopwood *et al.* (1985). Genomic DNA was purified according to Hintermann *et al.* (1981). *In vitro* DNA manipulations were done by standard methods. For the Southern blot and hybridization (Southern, 1975), genomic DNA digests were separated on 0.75% (w/v) agarose gels, blotted to Hybond N membranes (Amersham) and probed with the gel-purified 4 kb cloned DNA fragment which had been nick-translated with 5'-[α - ^{32}P]deoxycytidine (0.3 μg ; $2-3 \times 10^7$ c.p.m. μg^{-1}). Following prehybridization and hybridization in 50% (v/v) formamide at 42°C the membranes were washed three times for 20 min at 65°C in 0.1% SDS, $2 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M-NaCl, 0.015 M-trisodium citrate, pH 7.0).

Transductions and transformations. Phage integration was detected as in Piret & Chater (1985), by selecting for antibiotic resistances carried by the phage vectors: thiostrepton resistance (*tsr*; 50 $\mu\text{g ml}^{-1}$), viomycin resistance (*vph*; 40 $\mu\text{g ml}^{-1}$). The gene library used to isolate the wild-type *bldB* region was that of Piret & Chater (1985), and consisted of *Pst*I genomic DNA fragments from *S. coelicolor* M145 (insert frequency approx. 88%, size range 2.5–6 kb) inserted into ϕ C31 KC401 (Harris *et al.*, 1983).

For the primary cloning experiment, mycelium of the recipient strain was spread on R2YE agar medium and infected by replica-plating with library phage plaques. (Prior to replica-plating, the *S. lividans* lawn was killed by exposure of the plaque plates to chloroform fumes for 30 min.) Following growth at 30°C for 5–6 d, transductants were selected by replica plating to R2YE medium containing viomycin and thiostrepton. For routine transductions, phages and mycelium of the recipient strains were mixed and plated on R2YE, incubated at 30°C for 5–6 d, replica plated to R2YE containing the appropriate antibiotic(s) and incubated for 6 d, after which colony morphology of transductants was scored by visual inspection.

Streptomyces protoplasting and transformations were done according to the procedures of Hopwood *et al.* (1985). *E. coli* transformations were as described by Tabak *et al.* (1979).

Results

Isolation of the *bldB* region

The strategy used to clone the wild-type *bldB* region was similar to that previously described for the isolation of *bldA* (Piret & Chater, 1985). The temperate phage vector

ϕ C31 KC401 (Harris *et al.*, 1983) was used in complementation tests with cloned DNA at single copy number level. In this work, complementation refers to the restoration of aerial mycelium and spore chain formation as determined by visual inspection of colonies and microscopic observation of coverslip impressions. Because ϕ C31 KC401 contains a deletion eliminating *c* gene (repressor) function and another deletion eliminating its *attP* site, the *bldB* mutant strain (J669; *bldB43*) used as the indicator for complementation had first to be lysogenized with a *c*⁺ *attP*⁺ ϕ C31 derivative (ϕ C31 KC301 was used). This lysogen could be transduced to form a viomycin-resistant double lysogen either by homologous recombination between the resident prophage and ϕ C31 KC401 or its derivatives or, much less frequently, by recombination between the cloned fragment and its homologue in the chromosome (insert-directed integration).

Mycelium of J669 (ϕ C31 KC301) spread on R2YE plates was infected by mixing with approximately 6000 plaques of the pooled gene library. Only about 500 transductants were obtained after replication on R2YE plus viomycin. This rather low efficiency was attributed to the difficulty of replica-plating the smooth, hard colonies formed by *bldB* strains and to the fact that the host strain does not allow ϕ C31 multiplication. However, about half of the transductants produced aerial hyphae as well as pigmented antibiotics. This large apparent proportion of potential wild-type *bldB* clones was attributed to the relative ease of transfer of Bld⁺ colonies by replica-plating, thereby enriching for the desired clones.

Examination of twelve representative Bld⁺ transductants by phase contrast microscopy revealed that they all produced aerial hyphae and spore chains indistinguishable from those of the wild-type. They also retained the auxotrophic markers (requirements for homoserine and cysteine) of J669. Phages forming clear plaques (since ϕ C31 KC401 is *c*⁻) released from the twelve lysogens were purified and their DNA analysed by restriction enzyme digestion. They shared a *Pst*I insert fragment of approximately 4 kb. Phages carrying either orientation of the insert were found and representatives were named ϕ C31 KC625 and ϕ C31 KC626. Upon retransduction of J669 (ϕ C31 KC301) with either ϕ C31 KC625 or ϕ C31 KC626, nearly all the colonies screened exhibited wild-type morphology. In Fig. 1, electron micrographs illustrate this complementation.

Complementation of other *bldB* mutants

In addition to *bld-43*, Merrick (1976) assigned the *bld-15*, *bld-17* and *bld-28* alleles to the *bldB* region. ϕ C31 KC301 lysogens of representative strains (J701, J704 and J703)

Fig. 1. Restoration of wild-type morphology to a *bldB* mutant lysogenic for a phage carrying wild-type *S. coelicolor* DNA sequences (ϕ C31 KC626). Scanning electron micrographs (at equal magnification) by Dr D. Studer. Samples were from 7-d-old colonies grown on R2YE agar containing viomycin and thiostrepton. (a) Parent strain (J650) double lysogen for ϕ C31 KC301 and ϕ C31 KC401 (the cloning vector); aerial hyphae and spore chains are formed. (b) *bldB43* Mutant (J669) lysogenic for ϕ C31 KC301 and ϕ C31 KC401; only substrate mycelium is formed. (c) *bldB43* Mutant (J669) carrying ϕ C31 KC301 and ϕ C31 KC626; aerial hyphae and spore chains are formed. Bar, 1 μ m.

were prepared and transduced with ϕ C31 KC625 and ϕ C31 KC626. Like J669, J701 and J704 were phenotypically complemented in this way: the vast majority of the transductants were Bld⁺. Thus *bld-43*, *bld-15* and *bld-17* appear to be alleles of the same gene or mutations in closely linked genes present on the 4 kb fragment.

The rare Bld⁻ colonies found among the complemented transductants probably arose through homogenization, which sometimes accompanies insert-directed integration of recombinant phages (via the cloned *bldB* region in this case; Piret & Chater, 1985). This provided

Fig. 2. (a) Gel electrophoresis of *Pst*I digests of total genomic DNA of *S. coelicolor* parent strain and *bldB* mutants, other *Streptomyces* species and more distantly related organisms. (b) Hybridization of the 4 kb *bldB* fragment. Lanes 1, 8, 9, 10 and 20; size standards of λ DNA digested with *Hind*III (23.1, 9.4, 6.6, 4.4, 2.3 and 2.0 kb). Lanes: 2, *S. coelicolor* J650 (parent); 3, J669 (*bldB*43); 4, J701 (*bld*-15); 5, J703 (*bld*-28); 6, J704 (*bld*-17); 7, J668 (*bldA*39); 11, *S. coelicolor* M145; 12, *S. lividans* 66; 13, *S. parvulus* ETH 2283; 14, *S. glaucescens* ETH 22794; 15, *S. scabies* ETH 3171; 16, *S. peucetius* ETH 3180; 17, *S. noursei* ETH 3027; 18, *B. thuringiensis* var. *israeliensis*; 19, *A. mediterranei* ATCC 13685. Hybridization was obtained in lanes 2 to 7 and lane 11.

a method for detecting *bldB* mutations carried by phages. Phages released from representative *Bld*⁻ transductants were purified. A ϕ C31 KC628 (*c*⁺ Δ *attP*) derivative carrying the *bld*-15 mutation (ϕ C31 KC628-*bld*-15) was used to move this mutation into a new genetic background, *S. coelicolor* J1501 (H. Yu & J. Piret, unpublished). The new strain, *S. coelicolor* P101, exhibited the typical *bldB* phenotype: smooth, hard colonies independent of the carbon source supplied in the medium. This was good evidence that the cloned DNA fragment carries the wild-type *bldB* region, rather than a *bldB* suppressor.

The *bld*-28 mutant J703 was not complemented by the cloned region. J703 (ϕ C31 KC301) transduced with ϕ C31 KC625 or ϕ C31 KC626 produced only very sparse aerial hyphae after prolonged incubation. It was possible that the cloned region includes more than one *bld* gene and that the sequences corresponding to the *bld*-28 allele are present but truncated at one end. To test this, ϕ C31 KC628 was used to transduce J703 by insert-oriented integration. However, no wild-type colonies were found among several hundred transductants screened. A Southern blot of *Pst*I-digested total genomic DNA isolated from the parental strain J650 and from each *bldB* strain was probed with the cloned sequences. Hybridization to a 4 kb fragment was obtained in all cases (Fig. 2, lanes 2 to 7). Thus the lack of *bld*-28 complementation could not be explained by a detectable rearrangement or deletion event in this region in J703.

Genetic mapping placed *bldB* in a region of the *S. coelicolor* linkage map which carries a number of other developmental loci, between *whiG* and *whiH* (Merrick, 1976). Thus the ability of ϕ C31 KC625, ϕ C31 KC626 or

ϕ C31 KC628 to complement representative *whiG* and *whiH* mutations (in J118 and J95, respectively) was tested. Neither *whi* strain regained wild-type colony development.

Localization of *bld*-15 and *bld*-17 on the cloned fragment

In experiments to define the cloned sequences required for *bldB* complementation, the 4 kb region as well as smaller restriction fragments were introduced into multicopy plasmid vectors and tested in the parent strain J650 and the mutants J669, J701 and J704. The phenotypes of the J669 (*bldB*43) transformants were difficult to evaluate since colonies carrying the plasmid vectors with or without cloned insert DNA produced a leaky phenotype for unknown reasons. However, the presence of the vectors had no effect on the morphologies of any of the other strains.

Derivatives of pMEA3 (Hintermann *et al.*, 1985), pIJ364 (Kieser *et al.*, 1982) or pIJ486/487 (Ward *et al.*, 1986) carrying the entire 4 kb fragment exhibited marked structural instability in J701, J704 or J650. Following growth on selective medium, the colonies formed by the transformants were extensively sectorized *Bld*⁺ and *Bld*⁻. Plasmid DNA isolation showed that they carried mixtures of plasmids of sizes smaller than expected. On the other hand, when the same ligations were used to transform *S. lividans* 66, the expected plasmid constructs were obtained and appeared structurally stable. When plasmid DNA propagated in *S. lividans* was introduced into *S. coelicolor*, plasmid deletions occurred once again. This instability was investigated further, as described in the next section.

J701 (*bld*-15) transformed with pNTB01, pNTB02 or

Fig. 3. Restriction endonuclease map of the cloned 4 kb *bldB* DNA and phenotypic complementation of *bld-15* and *bld-17* strains. Sites were mapped using single and double digests of pIJ2154 and pIJ2155 (4 kb *bldB* *Pst*I fragment in pBR322 in each orientation). The orientation of the map shown is for the fragment in ϕ C31 KC401 as drawn by Hopwood *et al.* (1985), producing ϕ C31 KC626. Specific restriction fragments, represented by the thick bars drawn beneath the restriction map, were introduced into the multicopy vectors indicated, producing the plasmids listed. The thin bar in pNVB18 represents a deleted region. *Phenotypic complementation was tested by transformation into J701 (*bld-15*) and J704 (*bld-17*) and comparison of colony morphology with control strains carrying the plasmid vector without insert. Bld⁺, wild-type morphology; Bld⁻, mutant morphology; Bld^{+/-}, mixture of Bld⁺ and Bld⁻ colonies; ND, not done. †Plasmid structural stability following growth of the transformants on selective medium: +, all transformants analysed carry a single plasmid species of the expected size; -, some or all transformants analysed carry mixtures of plasmid species smaller than expected. ^aAll transformants analysed carry plasmid DNA smaller in size than expected. ^bBld⁺ transformants carry a single plasmid species of the expected size, Bld⁻ strains harbour a mixture of plasmids smaller in size than expected. ‡Complementation pattern: +, fragment complements mutant phenotype; -, fragment does not complement mutant phenotype; ?, complementation uncertain since all transformants examined carry plasmid species smaller in size than expected; +/-, transformants are a mixture of Bld⁺ and Bld⁻ colonies; ND, not done.

pNMH3, which carry a 1.5 kb *Sph*I–*Sst*I fragment originating from the centre region of the original cloned insert (Fig. 3; T. Breidenbach & V. Bernan, unpublished results), produced a mixture of Bld⁺ and Bld⁻ colonies in a ratio of about 2:1. In this case, isolation of plasmid DNA showed that both types of transformants carried plasmids of the expected sizes. Retransformation of J701 with plasmid DNA from each colony type resulted in a similar mixture of Bld⁺ and Bld⁻ colonies in each case. Deletion of a 0.4 kb *Bam*HI fragment internal to the 1.5 kb region abolished complementation: all pNVB18 transformants were Bld⁻ (V. Bernan, unpublished results). A representative sporulating J701 pNMH3 strain was cured of its plasmid by growth in the absence of antibiotic (thiostrepton) selection and screening for thiostrepton sensitivity. The seven strains obtained in this way were morphologically Bld⁻, suggesting that J701 complementation does not require integration of the cloned sequences but apparently occurs *in trans*. In no case could pNMH4, pNMH5 or pNMH6 complement the Bld⁻ morphology of J701.

The complementation pattern for the *bld-17* mutant, J704, differed from that for J701 (*bld-15*). J704-carrying pNTB01 or pNTB02 did not regain wild-type colony

morphology. Upon prolonged incubation the transformants developed very sparse aerial hyphae. A larger DNA fragment of about 2.3 kb which includes the 1.5 kb *bld-15*-complementing region was needed to complement J704. Although efforts to complement *bld-17* with a stable plasmid construct have so far proved unsuccessful (see below), J704 carrying pNMH1 or pNLZ34 (Fig. 3) produced a small proportion of colonies with wild-type morphology. Examination of the plasmid DNA carried by the transformants revealed, in both cases, that Bld⁺ strains carried plasmids of the expected sizes whereas Bld⁻ strains contained plasmid populations which were a mixture of sizes smaller than expected.

Localization of DNA sequences affecting plasmid stability

In addition to restoring wild-type colony morphology to J701, the presence of the 1.5 kb *Sph*I–*Sst*I fragment in pIJ702 leads to plasmid structural instability or strong inhibition of the growth of the host strain. The pattern is shown in Fig. 3. J701 transformants carrying pNMH1 or pNMH3 formed a mixture of Bld⁺ and Bld⁻ colonies. However, plasmids isolated from these strains revealed two different situations. In Bld⁺ strains, pNMH1

appeared to be intact whereas in bald transformants the plasmid DNA was composed of mixtures of species smaller in size than expected. On the other hand, as noted earlier, both Bld⁺ and Bld⁻ pNMH3 transformants carried a single plasmid species of the expected size. However, the growth of pNMH3 transformants was significantly slower than that of the parent or of J701 carrying pIJ702 without insert. Although pNMH4, pNMH5 and pNMH6 did not complement J701, pNMH4 and pNMH6 transformants carried apparently intact plasmid DNA of the expected sizes whereas pNMH5 suffered structural deletions at a high frequency. In addition to pNLZ34, a number of subclones of the *bldB* region in pIJ364 were also constructed and tested in J701 and J704. A pattern of structural instability and growth inhibition similar to that seen with the pIJ702 derivatives occurred (data not shown).

Comparison of the inserts carried by the various pIJ702 and pIJ364 derivatives implicates the 0.6 kb *Bgl*II-*Sph*I-*Bam*HI insert fragment shared by pNMH1, pNMH3, pNMH5 and pNLZ34 in the plasmid structural instability and growth inhibition phenomena. The same pattern of plasmid instability and growth inhibition occurred when the various subclones were introduced into other *S. coelicolor* strains such as the parental strain J650 or the prototroph M145. However, in *S. lividans* all of these plasmids were structurally stable and the host growth rate was normal.

Absence of the cloned *bldB* sequences in other species

A Southern blot of genomic DNA isolated from several *Streptomyces* species as well as from some more distantly related micro-organisms, was probed with the cloned *bldB* fragment under stringent conditions (Fig. 2, lanes 11 to 19). Strains of *S. parvulus*, *S. glaucescens*, *S. scabies*, *S. peucetius*, *S. noursei*, *S. lividans*, *A. mediterranei* and *B. thuringiensis* var. *israeliensis* were used. Unexpectedly, none showed homology with the *bldB* sequences. This was particularly surprising for *S. lividans*, which is closely related to *S. coelicolor*.

Discussion

The cloned 4 kb wild-type *bldB* region restores apparently wild-type aerial hyphae and spore chain formation to the *bld-15*, *bld-17* and *bld-43* alleles mapped by Merrick (1976). Complementation by these sequences by integration into a resident prophage at the host *att* site indicates that the corresponding wild-type sequences are complete on the cloned DNA fragment and dominant over the mutant alleles. Plasmid subcloning experiments to

localize *bldB*-complementing sequences showed that *bld-15* is complemented by a 1.5 kb fragment while additional adjacent sequences of about 0.8 kb must also be present (2.3 kb total) for *bld-17* complementation. *bld-15* and *bld-17* may lie in separate genes. Alternatively, J704 may carry two *bld* mutations. The testing of the *bld-43* strain J669 was prevented by its leaky phenotype when carrying the plasmid vectors with and without insert DNA.

Phenotypic complementation of J701 (*bld-15*) using stable multicopy plasmid (pIJ486/7) derivatives occurred in about 70% of the transformants. This suggested that the cloned genes corresponding to this mutation might be truncated and that recombination with the homologous chromosomal region was required for complementation. However, plasmid curing of a Bld⁺ J701 pNMH3 strain produced only bald colonies, indicating that integration is not required. Some other explanation, involving the nature or function of the *bld-15* gene, copy number effects or some physiological variability in the transformants, must be considered to explain the mixture of Bld⁺ and Bld⁻ morphologies.

In crosses between *bldB* mutants, Merrick (1976) detected a low frequency of wild-type recombinants in some cases. Upon lysogenization with ϕ C31 KC625 or ϕ C31 KC626, the *bld-28* strain J703 (ϕ C31 KC301 lysogen) remains bald. One possibility is that *bld-28* is a dominant allele of wild-type *bldB*. However, no Bld⁺ Vio^r lysogens arising by homogenotization were found among the ϕ C31 KC628 transductants. The failure to find Bld⁺ transductants also rules out the possibility that the sequences corresponding to *bld-28* are truncated on the cloned fragment. ϕ C31 KC628 integrates by homology with the cloned insert and, at least occasionally, should have generated a wild-type gene during recombinational integration (Piret & Chater, 1985). Therefore *bld-28* is probably not a *bldB* allele. Champness (1988) isolated three additional *S. coelicolor* *bld* mutants which she assigned to the *bldB* region in mating experiments. She reported that two alleles, *bld-112* and *bld-186*, were complemented by ϕ C31 KC628, while *bld-249* was not. The *bld-249* transductants formed sparse aerial mycelium and no spore chains upon prolonged incubation, a phenotype analogous to that of J703 (*bld-28*) transductants. Thus *bld-28* and *bld-249* appear to represent at least one previously unrecognized additional *S. coelicolor* *bld* gene which maps near, but is not part of, the cloned region. Tentatively, we propose to name this locus *bldI*. The phenotypes of these mutants, like those of *bldB* strains, are unaffected by the carbon source provided in the growth medium. Cloning of the *bld-28* and *bld-249* DNA region(s) will permit the characterization of this new developmental locus and its possible relationship with *bldB*.

Derivatives of the multicopy plasmids pIJ702 or pIJ364 carrying inserts from the cloned *bldB* region were structurally unstable or strongly inhibited the growth of *S. coelicolor* in a number of cases. The presence of the 0.6 kb fragment containing the *Bgl*II-*Sph*I-*Bam*HI cluster of sites (Fig. 3) appears to be responsible for these phenomena. Deleted derivatives of pNMH1, pNMH5 and pNLZ34 occurred at high frequencies. pNMH3 was structurally stable but its presence markedly inhibited the growth of *S. coelicolor*. On the other hand, although they are also derived from pIJ101 (Kieser *et al.*, 1982), pIJ486/487 constructions stably maintained the same *bldB* sequences. The reason for this stability may be related to the presence of a transcriptional terminator adjacent to the multiple cloning site in pIJ486/487 (Ward *et al.*, 1986). Analysis of the DNA sequence of this region may reveal clues as to the basis for these phenomena.

Examination of colony morphology by coverslip impressions or electron microscopy showed that the cloned *bldB* region restores apparently wild-type morphological differentiation to complemented mutants. Mendez & Chater (1987) reported evidence of 'hyper-sporulation' when the wild-type *S. coelicolor* *whiG* region was present in two or more copies. *whiG* is known to encode an RNA polymerase sigma factor required for spore chain formation (Chater *et al.*, 1989). Such copy number effects were not observed in the case of *bldB*.

Unexpectedly, DNA with significant homology to the *bldB* region was absent in other *Streptomyces* species, including the closely related strain *S. lividans* 66. (This may explain the stability of the cloned sequences in multicopy plasmids in this species.) It is possible that although *bldB* appears to be unique to *S. coelicolor* at the DNA level, gene product(s) functionally homologous to those which it encodes are produced in other *Streptomyces* species. There may be processes involved in *Streptomyces* morphological development which are species-specific. Indeed, different species of *Streptomyces* are known to produce and to respond to specific regulatory factors controlling morphological and physiological (secondary metabolism) differentiation (Khokhlov, 1988). *bldB* may play a necessary role in such a specific mechanism in *S. coelicolor* colony development.

Unlike the two previously characterized *S. coelicolor* developmental regions, *bldA* and *whiG*, which encode single gene products (Lawlor *et al.*, 1987; Chater *et al.*, 1989), *bldB* is composed of at least two loci. We have isolated and are analysing one of these. It complements four *bldB* mutants and corresponds to at least one *bld* gene. DNA sequencing and analysis of gene expression in the cloned region are in progress with the objective of identifying the nature and function of the gene(s) involved. Efforts are also under way to isolate the second

bldB region, now *bldI*, by complementation of the two remaining '*bldB*' mutant strains.

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