The bldB Gene Encodes a Small Protein Required for Morphogenesis, Antibiotic Production, and Catabolite Control in Streptomyces coelicolor

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Mutants blocked at the earliest stage of morphological development in Streptomyces species are called bld mutants. These mutants are pleiotropically defective in the initiation of development, the ability to produce antibiotics, the ability to regulate carbon utilization, and the ability to send and/or respond to extracellular signals. Here we report the identification and partial characterization of a 99-amino-acid open reading frame (ORF99) that is capable of restoring morphogenesis, antibiotic production, and catabolite control to all of the bldB mutants. Of the existing bld mutants, bldB is of special interest because the phenotype of this mutant is the most pleiotropic. DNA sequence analysis of ORF99 from each of the existing bldB mutants identified base changes either within the coding region of the predicted protein or in the regulatory region of the gene. Primer extension analysis identified an apparent transcription start site. A promoter fusion to the xylE reporter gene showed that expression of bldB is apparently temporally regulated and that the bldB gene product is involved in the regulation of its own expression.

Streptomyces grow vegetatively as a branching mycelial mass. Presumably, in response to nutrient depletion, these multicellular bacteria initiate a complex morphogenetic program that involves both structural and biochemical adaptation. The first visible evidence of the initiation of development is the erection of aerial hyphae. As development proceeds, these hyphae coil and septate into uninucleoid compartments that give rise to spores, and the substrate mycelium begins to lyse. As these morphological changes occur, the organism produces antibiotic activity (8). Mutants blocked at the earliest stages of morphogenesis, those that fail to make aerial hyphae, are called bld mutants (9, 27). These mutants are also defective in antibiotic production (7, 8), catabolite repression (30), and cell-cell signaling (38, 39). Most of what we know about these genes and their roles in development comes from the study of bld mutants of Streptomyces coelicolor. At least 10 bld loci, bldA, bldB, bldC, bldD, bldF, bldG, bldH, bldI, bldK, and bld261, have been found in S. coelicolor (7, 9, 27, 28, 38, 39), and the highly pleiotropic phenotype of these mutations suggests, a priori, that they identify genes involved at an early stage in the initiation of development.

Only two bld genes, bldA and bldK, have been characterized at the molecular level. The bldA alleles reside in a gene for a leucyl-tRNA that recognizes the UUA codon (21). UUA is a rare codon in S. coelicolor, and it has been suggested that this tRNA is involved in the translation of regulatory genes involved in antibiotic production and morphogenesis (12, 23, 24). While the level of bldA expression apparently increases at the initiation of development, the gene product is clearly present and active during vegetative growth (36). The role of this tRNA in catabolite control or in generating or responding to morphogenic signals remains unclear. The bldD gene has recently been cloned and characterized and is described in the accompanying report (10).

An important property of bld mutants is that they exhibit extracellular complementation (38, 39). This complementation apparently results from the diffusion of substances from one strain to another when patches of cells are grown in close proximity to each other on agar plates. Willey et al. (39) have suggested that the pattern of complementation observed among different mutant classes defines an intercellular signaling pathway involving as many as four extracellular factors. They postulated that the products of the bld genes are either directly or indirectly involved in the generation or uptake of extracellular signaling molecules such as SapB (39). Nodwell et al. (28) have recently shown that the bldK locus consists of five adjacent open reading frames (ORFs) that specify homologs of the subunits of the oligopeptide-permease family of ATP-binding cassette (ABC) membrane-spanning transporters. It has been inferred that bldK is an oligopeptide transporter and is perhaps responsible for the import of an extracellular signal required for the initiation of morphogenesis.

One of the most intriguing but poorly understood aspects of the bld phenotype is that growth on poor carbon sources is sufficient to restore partially the morphological and antibiotic defects of most of these mutants (7). An important exception is bldB (7, 27, 30). When grown on minimal medium agar plates containing glucose as the carbon source, bld mutants fail to erect aerial hyphae and are also defective in antibiotic production. When the mutants are grown on minimal medium containing mannitol, however, aerial hyphae and spore production are partially restored. While growth on mannitol partially rescues the morphogenetic defect of bldA mutants, the cells remain deficient in antibiotic production (7, 27). In contrast, growth on mannitol rescues both sporulation and antibiotic production in bldH mutants (7). The most severely affected of the bld mutants, bldB, remains both morphologically and physiologically defective, failing to sporulate or produce antibiotics, regardless of carbon source (7, 27, 30).

In recent work, we have shown that bldA, bldB, bldC, bldD,
bldG, and bldH mutants are defective in the regulation of the galP1 promoter, a glucose-sensitive and galactose-dependent promoter that directs expression of the galactose utilization operon, and that the bldB mutant is globally deregulated for catabolite control (30). Screens for mutants defective in catabolite control identified mutants that were at once resistant to glucose repression, defective in the regulation of antibiotic production (they overproduce antibiotics precociously), and bld (30). These observations strongly suggest that there is a direct connection between the regulation of carbon utilization and the initiation of morphogenesis in streptomycetes.

Of the existing bld mutants, bldB mutants are of special interest because their phenotype is the most pleiotropic. bldB mutants are completely defective in antibiotic production (7) and apparently globally defective in the regulation of carbon utilization (30), fail to initiate morphological development (27), and are the only bld mutants whose phenotype is not rescued by growth on poor carbon sources (7, 27, 30). Interestingly, bldB mutants also do not fit into the hierarchical signaling cascade proposed by Willey et al. (39). Harasym et al. (16) identified a 4-kb fragment from S. coelicolor (GenBank accession no. U28930) and showed that it complemented some of the bldB mutant alleles. They concluded from complementation analysis that the bldB locus contained at least two genes involved in morphological development (27), and are the only bld mutants whose phenotype is not rescued by growth on poor carbon sources (7, 27, 30). Interestingly, bldB mutants also do not fit into the hierarchical signaling cascade proposed by Willey et al. (39).

bldB and bldH mutants are involved in morphological development. Here we report the cloning and sequencing of a portion of that fragment that complements all of the existing bldB mutant alleles. DNA sequence analysis of the complementing fragment identified a single small ORF capable of encoding a 99-amino-acid protein, and we suggest that this ORF is the bldB gene. DNA sequence analysis of this ORF from each of the existing bldB mutants identified base changes either within the coding region of the putative protein or in the regulatory region of the gene. Two of the mutations, bldB28 and bldB17, lie in the -10 region of the bldB promoter. bldB249 contains a base change in the putative ribosome binding site. The bldB112 mutation introduces a stop codon and would result in a truncated protein of 71 amino acids. The bldB186 mutation creates a frameshift and would result in a slightly larger protein of 134 amino acids. Two mutations, bldB15 and bldB43, identify the same tyrosine residue at position 21 to be important for bldB function. Primer extension analysis of the bldB transcript identified an apparent transcription start site. Analysis of bldB expression by using a bldB promoter fusion to the yE* reporter gene revealed that expression of bldB in wild-type cells is low during vegetative growth and increases as the cells enter stationary phase. bldB expression is apparently deregulated in a bldB mutant, suggesting that the bldB gene product is involved in the regulation of its own transcription.

TABLE 1. Strains of S. coelicolor containing bldB mutant and wild-type alleles

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype or phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>J701</td>
<td>bldB15 mthB2 auraA7 NF SCP2*</td>
<td>27</td>
</tr>
<tr>
<td>J703</td>
<td>bldBb22b2 auraA7 NF SCP2*</td>
<td>27</td>
</tr>
<tr>
<td>J704</td>
<td>bldBb17 mthB2 auraA7 NF SCP2*</td>
<td>27</td>
</tr>
<tr>
<td>J669</td>
<td>bldBb43 mthB2 cysD18 auraA17 NF SCP2*</td>
<td>27</td>
</tr>
<tr>
<td>C112</td>
<td>bldB112 hisA1 uaaA1 strA1 SCP2* SCP1* Pgl*</td>
<td>7</td>
</tr>
<tr>
<td>C186</td>
<td>bldB186 hisA1 uaaA1 strA1 SCP2* SCP1* Pgl*</td>
<td>7</td>
</tr>
<tr>
<td>C249</td>
<td>bldB249 hisA1 uaaA1 strA1 SCP2* SCP1* Pgl*</td>
<td>7</td>
</tr>
<tr>
<td>KC628</td>
<td>ΔC31 derivative; bldB+ C* δmP Vph+</td>
<td>16</td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Bacterial strains and plasmids.** S. coelicolor A(3)2 and S. lividans 1326 (17) were used as hosts for plasmids and plasmages. The bldB strains used are described in Table 1. Subcloning of phage fragments or PCR-generated products was carried out according to standard E. coli techniques (25). KC628 (16) is a derivative of the S. coelicolor phage fC31, Escherichia coli DH5αMCR (Bethesda Research Laboratories) was used to prepare DNA for transformation into Streptomyces.

**Plasmid constructions.** Phage KC628, containing a 4-kb PstI fragment from the S. coelicolor chromosome (Fig. 1), was provided by Jacqueline Piret (16). Phagemid pMP1.6 was generated by cloning a 1.6-kb BglII-Sacl fragment from KC628 into the high-copy-number Streptomyces plasmid pJ702 (17). This plasmid contains approximately 380 nucleotides upstream of the ORF fragment translational start site, all of ORF99, and a portion of an adjacent ORF which is oriented on the opposite strand in the opposite direction. PCR primers were designed to amplify either a 573-bp fragment (upstream primer 5′-CCACCACTCGCGCCCAGATTTAATGTC-3′; downstream primer 5′-TTGAGATTCCGTCGTCGTAATTCTAC-3′) that contains ORF99 as well as approximately 176 bp upstream or a 777-bp fragment (upstream primer 5′-GGCGGAGATCTGTCAGGTCCTG-3′; downstream primer 5′-TCTCGTCGTGCTCATCTCGGGTAC-3′ that included an additional 204 bases upstream. The 573-bp PCR fragment was cloned into the PstI/BglII sites of pJ702, generating pMP753. Ligation mixtures were transformed into S. lividans 1326 and screened for disruption of the melanin gene contained on the vector. Plasmids were isolated from this strain and analyzed by restriction digestion before transformation into either A(3)2 or the bldB mutant strains. The 777-bp PCR fragment contained engineered BglII sites on either end and was cloned into the BamHI site of pX4E4, which contains an SCP2 replicon, generating pMP777. A BglII-BamHI fragment containing the 5′ end of ORF99 and upstream region was cloned into pX4E4 (19), generating a promoter fusion to the yE* reporter gene, designated pX8E5.

**Media and growth conditions.** Minimal medium plates supplemented with the appropriate amino acids were made as described previously (4). Thiostrepton (50 μg/ml) was included for selection of plasmids. Prior to plating of cells, the surface of the agar was covered with a sterile cellophane disc (19) to allow collection of cells without agar contamination for RNA isolation. Plates were incubated at 30°C. Genomic DNA was isolated from cells grown in liquid YEME media and increases as the cells enter stationary phase. bldB production (they overproduce antibiotics precociously), and bld (30). Screens for mutants defective in catabolite control identified mutants that were at once resistant to glucose repression, defective in the regulation of antibiotic production (they overproduce antibiotics precociously), and bld (30). These observations strongly suggest that there is a direct connection between the regulation of carbon utilization and the initiation of morphogenesis in streptomycetes.

![Figure 1](http://jb.asm.org/Downloaded)
and the entire reaction mixture was loaded onto gels. The corresponding sequencing ladder primer extension product shown in Fig. 3 was generated by using the primer 120 bases downstream of the ATG (5′-GGCTTTGACGTCCTC-3′) and a third oligonucleotide located approximately the translation start site (5′-GTCCTCGTCCGGCACCTGGGC-3′). The base changes identified in various bldB mutant strains are shown, with the predicted peptide sequence indicated beneath the coding region.

**RESULTS**

Identification of the bldB gene. In 1990, Harasym et al. (16) reported that a 4-kb PsI fragment of *S. coelicolor* DNA (shown in Fig. 1) contained in phage KC628 rescued the morphological defect of some of the known bldB mutants. They also showed that deletion of an internal BamHI fragment within the PsI fragment resulted in loss of rescue of the bldB phenotype. We cloned the portion of the PsI fragment, an internal

$BglII-SacI$ fragment, that included the region identified by this deletion on the high-copy-number plasmid pMP1.6 (Fig. 1) and determined the DNA sequence. Sequence analysis of this 1.6-kb fragment revealed the existence of one small ORF capable of encoding a protein of 99 amino acids, which we refer to as ORF99, and part of another ORF. A 573-bp fragment containing only ORF99 and approximately 176 bp upstream of the translational start site was amplified by using PCR from wild-type *S. coelicolor* genomic DNA. This PCR product was cloned into the high-copy-number Streptomyces plasmid pJ702, generating plasmid pMP573 (Fig. 1). pMP573 was then transformed into protoplasts of the bldB mutant strains listed in Table 1. Nine bldB mutant alleles, bldB15, bldB16, bldB17, bldB43, bldB57, bldB59 (27), bldB112, bldB186, and bldB249 (7), have been described. bldB57 and bldB59 did not survive storage and were not recovered from the John Innes strain collection (8a).

In our experiments, KC628 completely complemented all of the existing bldB mutants except J703 (bldB28) and C249 (bldB249). Complementation of either J703 or C249 with KC628 resulted in rescue only on mannitol. Sporulation on mannitol is characteristic of a *bldA* mutant. The fact that complementation of J703 or C249 resulted in a *bldA* phenotype suggested that these strains might contain two mutations. Leskiw and Mah (22) showed that while there is no mutation in the *bldA* gene of J703, the level of *bldA* transcript in this strain is greatly reduced. It is likely, therefore, that J703 contains two mutations, one in the bldB gene (Fig. 2) and a second mutation that affects bldA expression. In recent experiments, C249 has also been shown to contain two mutations, one in bldB (Fig. 3) and another in bldA (22).

Mutation analysis of the remaining bldB mutants, J701, J704, J669, and J703, was performed as described previously (19). Strains containing pMP573 after growth for 6 days (lane 5), 2 days (lane 6), or 1 day (lane 7); and A3(2) after growth for 3 days (lane 8), 2 days (lane 9), or 1 day (lane 10).
a strain that includes SCP1, the copy number is considerably lower than for strains that lack SCP1. Since the bldB mutant strains C112 and C186 lack SCP1, it is possible that the copy number of pMP573 was very high in these strains and the apparent failure of pMP573 to complement these mutants was actually due to plasmid instability or cell death. In an attempt to address this possibility, ORF99 was cloned into an SCP2, single-copy-number replicon to generate plasmid pMP777. pMP777 did rescue both C112 and C186. We suggest that the complementation observed in these strains results from the ORF99 gene product.

Physical mapping of the bldB mutations. To examine the sequence of ORF99 in the existing bldB mutants, we generated a 573-bp PCR fragment from each of the bldB strains as well as the wild-type strain and determined the DNA sequence. The sequence of ORF99 in individual bldB mutants is shown in Fig. 2. Three of the mutations, bldB28, bldB17, and bldB249, map just upstream of the predicted ORF. bldB249 contains a base change in the putative ribosome binding site. The bldB112 mutation introduces a stop codon and would result in a truncated protein of 71 amino acids. The bldB186 mutation creates a frameshift and would result in a slightly larger protein of 134 amino acids. Two mutations, bldB15 and bldB43, identify the same tyrosine residue at position 21 to be important for bldB function. We conclude from these data and the genetic complementation analysis that ORF99 encodes the bldB gene.

 Primer extension analysis of bldB RNA. To identify the site of transcription initiation for bldB, primer extension experiments were performed. As shown in Fig. 3, bldB-specific reverse transcripts were easily detected in strains containing the wild-type bldB gene on either of the high-copy-number plasmids pMP1.6 and pMP573. Three different oligonucleotide primers were used in these experiments, and in all cases, one of four G residues located 37 bp upstream of the apparent ATG translation start codon was identified as the transcription start site (Fig. 2). The same start site was identified in assays using RNA isolated from wild-type S. coelicolor with no plasmid. While this chromosomal transcript of bldB was readily detectable in these experiments and is the same size as the transcript generated from the plasmid copy, the amount of transcript detected was considerably less than in cells containing the bldB gene on plasmids. While these primer extension reactions were not rigorously quantitative, we used the same amount of RNA in each reaction and observed significantly stronger signals from cultures grown for 2 days than from those grown for 1 day. The results of this primer extension analysis place two of the bldB mutations, bldB28 and bldB17, in the -10 region of the bldB promoter.

 Detection of bldB promoter activity by using a transcriptional fusion to the xyle reporter gene. A DNA fragment containing sequences from 380 nucleotides upstream to 125 nucleotides downstream of the apparent transcription start site of bldB was subcloned upstream of a promoterless copy of the xyle reporter gene in the low-copy-number plasmid pXE4 (19), resulting in plasmid pXE85 (Fig. 1). The xyle gene product, catechol 2,3-dioxygenase, oxidizes catechol and results in a color change (clear to yellow) in colonies expressing the xyle gene. Visual inspection of colonies, grown on either glucose or mannitol as the carbon source, that contained the bldB-xyle fusion showed intense yellow color after exposure to catechol, while colonies containing only the xyle reporter gene with no promoter remained white (data not shown).

 Expression from the bldB promoter was examined by using the bldB-xyle fusion contained in pXE85. The quantitative catechol dioxygenase assays in Fig. 4 show that expression of the bldB-xyle fusion in wild-type cells is low during vegetative growth and increases as cells enter stationary phase between 2 and 3 days of growth. This result is in good agreement with the primer extension analysis of bldB RNA shown in Fig. 3. Expression of the bldB-xyle fusion in a bldB mutant, however, is increased and apparently deregulated. This is true regardless of whether cells are grown on glucose or mannitol as the carbon source (Fig. 4). We conclude from these data that the apparent temporal expression of bldB is dependent on bldB itself and that bldB is likely involved in its own regulation.

 Structural analysis of the bldB gene product. Neither the DNA sequence of the bldB gene nor the 99-amino-acid protein sequence deduced from the DNA sequence resembles any sequence in the GenBank database. When sequence comparisons fail to identify homologs, it is sometimes possible to deduce the likely fold that the putative protein sequence can adopt by threading that sequence onto known protein folds and scoring the resulting model for correctness (3). (Several hundred unique protein fold patterns have been identified by X-ray crystallography from more than a thousand different proteins.) A measure of physical stereoechemical compatibility is obtained by assessing whether the fit of each residue of the protein of interest to the atomic coordinates of the corresponding residue of the protein fold (e.g., determining whether the fold places the hydrophobic groups in the interior and charged groups on the surface, whether it places the helix-forming residues in helices, etc.). Experience with this method suggests that false positives are not common (13), but it is easy to miss a correct fold. High scores are worth using as the basis for further experiments.

 The bldB sequence was threaded (29a) onto all of the protein folds in the Brookhaven Protein Data Bank, using the algorithm of Bowie et al. (3). The three highest-scoring folds were all DNA binding proteins containing a helix-turn-helix DNA binding motif: the E. coli catabolite gene activator protein, 434 Cro protein, and λ repressor. In every case, the C-terminal region of bldB threaded well onto the helix-turn-helix region of each of these DNA binding proteins. These scores are a prediction and do not in any way prove that bldB is a DNA binding protein. This analysis does, however, suggest that experiments to test the possibility that bldB is a DNA binding protein are worthwhile. Several amino acids that could be important for protein-DNA interactions are also suggested.

FIG. 4. Expression from the bldB-xyle fusion in wild-type and bldB mutant strains. The histogram shows results of catechol dioxygenase assays (specific activity) from cells grown for 1, 2, or 3 days on either glucose or mannitol as the carbon source. Expression of the bldB-xyle fusion was examined in an S. coelicolor wild-type strain A3(2) and J701, which contains the bldB15 allele. Specific activity is expressed as the rate of change in optical density at 375 nm per minute per milligram of protein. Duplicate assays gave nearly identical results and so error bars are not visible.
by this analysis. These include glutamic acid residues at positions 62 and 69, a threonine at position 63, and an arginine at position 66.

DISCUSSION

We report the identification and partial characterization of the bldB gene of S. coelicolor. Genetic complementation analysis using subcloned portions of a 4-kb fragment of the S. coelicolor chromosome (16) identified a small ORF, ORF99, that restored sporulation to all of the known bldB mutants. Cloning and sequencing ORF99 from wild-type S. coelicolor and each of the bldB mutants identified base changes associated with this ORF in all of the existing bldB mutants. Three bldB alleles contained base changes in the S’ untranslated region of the gene, two contained changes in the −10 region of the promoter, and one contained changes in the putative ribosome binding site of ORF99. One allele contained a frameshift in ORF99, one contained a stop codon within ORF99, and two independently isolated bldB mutants contained base changes that would change the same tyrosine residue in the amino-terminal portion of ORF99 to either a leucine or a cysteine. We conclude from these data that ORF99 is the bldB gene.

Primer extension analysis identified an apparent transcription start site for bldB, placing base changes in two of the bldB mutant alleles in the −10 region of the promoter. The examination of bldB-specific reverse transcripts indicated that the level of bldB RNA is low during vegetative growth and increases at the initiation of development. Similarly, the level of expression of a bldB-xylose fusion was low level during vegetative growth and increased at the initiation of development. Taken together, these observations suggest that expression of bldB is temporally regulated.

Expression of the bldB-xylose fusion was increased and apparently deregulated in strains containing a bldB mutation, suggesting that the bldB gene product is required for regulation of bldB transcription. Interestingly, the level of bldB expression is higher in wild-type cells grown on glucose than in cells grown on mannitol. While there are no data to suggest that the bldB protein interacts directly with its own promoter, the bldB promoter is clearly a target of bldB activity. Structural analysis using protein threading of the predicted bldB peptide suggests the presence of a helix-turn-helix motif (5) in the carboxyl terminus with similarity to those of known DNA binding proteins. The BldB protein may function mechanistically in a manner similar to that of the AbrB protein of Bacillus subtilis. The AbrB protein plays a key role in the expression of genes during the transition between vegetative and stationary-phase growth of B. subtilis. The AbrB protein consists of only 96 amino acids and has been shown to bind its own promoter (35) and repress its own synthesis during vegetative growth (34). Unlike bldB mutations, however, AbrB mutations alone do not result in loss of sporulation; thus, while the mechanisms of binding and autoregulation may be the same, the biological functions of these proteins are clearly different.

One of the most interesting discoveries in this analysis of bldB is that two independently isolated bldB alleles identify the same residue, a tyrosine at position 21, to be important for bldB function. Tyrosine residues serve a number of roles in protein structure and function. Tyrosine residues are often important mediators of protein-protein interactions. Tyrosine is different from other hydrophobic, aromatic amino acids like phenylalanine and tryptophan in that its phenolic hydroxyl group is a strong hydrogen bond donor and acceptor. Consequently, tyrosines are often found on the surface of globular proteins or are partially buried with the hydroxyl group available for intermolecular hydrogen bonding (32). The tyrosine at position 21 may facilitate multimerization of BldB proteins for the kind of cooperative binding of DNA seen for AbrB which binds DNA as a hexamer (34). Alternatively, this tyrosine may facilitate protein-protein interaction between BldB and other proteins in the regulation of gene expression.

Tyrosine residues are also common sites for regulatory covalent modification, and these modifications, because they introduce charged groups, can act as switches either to create or abolish protein-protein contacts or to change the intramolecular conformation of proteins and thereby alter their activities. One intriguing possibility is that BldB is phosphorylated at tyrosine 21, and this phosphorylation event is required for the activity of bldB. While tyrosine phosphorylation in prokaryotes is not common, tyrosine residues are often the sites of phosphorylation in eukaryotic proteins. Waters et al. (37) demonstrated that eukaryotic protein kinase inhibitors blocked morphogenesis in Streptomyces and that the profile of tyrosine-phosphorylated proteins changed as Streptomyces initiated morphogenesis. Eukaryotic-type tyrosine kinases have also been implicated in signal transduction pathways affecting cell differentiation, pathogenicity, and secondary metabolism in a number of other bacteria, including Myxococcus xanthus (14, 15) and Salmonella typhimurium (29). The SpoOA protein, which plays an important role in the initiation of sporulation in B. subtilis, serves as an example of how phosphorylation activates regulatory proteins. Aspartate-phosphorylated SpoOA protein is a transcriptional activator required for the transcription of genes involved in the initiation of sporulation in B. subtilis (6). Unphosphorylated SpoOA is inactive as a transcriptional activator, and the regulation of the phosphorylation of SpoOA a key component of development.

The bldB gene is also clearly involved in catabolite repression in Streptomyces. Pope et al. (30) showed that bldB mutants are relieved of glucose repression of the galactose, glycerol, and agar utilization operons and that complementation of a bldB mutant with the wild-type bldB allele restores at once sporulation, antibiotic production, and glucose repression to these mutants. Unlike bldB mutants, unlike bldD mutants, the profile of tyrosine-phosphorylated proteins changed as Streptomyces initiated morphogenesis. Eukaryotic-type tyrosine kinases have also been implicated in signal transduction pathways affecting cell differentiation, pathogenicity, and secondary metabolism in a number of other bacteria, including Myxococcus xanthus (14, 15) and Salmonella typhimurium (29). The SpoOA protein, which plays an important role in the initiation of sporulation in B. subtilis, serves as an example of how phosphorylation activates regulatory proteins. Aspartate-phosphorylated SpoOA protein is a transcriptional activator required for the transcription of genes involved in the initiation of sporulation in B. subtilis (6). Unphosphorylated SpoOA is inactive as a transcriptional activator, and the regulation of the phosphorylation of SpoOA a key component of development.
carbon sources. There are other differences. *S. coelicolor* mutants that are resistant to 3-aminobenzamide or 3-methoxybenzamide are defective in ADP-ribosylation (33). These mutants are also defective in morphogenesis. In their study of such mutants, Shima et al. (33) showed that the patterns of ADP-ribosylated proteins in various *bld* mutants are different. In particular, the patterns detected in *bldB* and *bldD* mutants are totally different.

So how does one explain the function of a protein that is required for such diverse activities as the initiation of morphogenesis, the regulation of catabolite control, the global production of antibiotics, and the production signals required for cell-cell communication? The highly pleiotropic nature of the *bldB* mutations suggests, a priori, that the *bldB* protein is required for an early event in the initiation of development in *Streptomyces*. We might argue that because *bldD* mutants are defective in the regulation of carbon utilization, the genes they identify play key roles in the complex process of cellular differentiation. It is difficult to know at this point how or, in fact, whether their genes products interact. As Willey et al. (39) point out, the cascade of signals that they observe for extracellular complementation is true for only one set of nutritional conditions, i.e., rich medium with glucose as the carbon source. Clearly, more information is needed about the molecular nature of the genes identified by the *bld* mutants as well as saturation mutagenesis of the *bld* loci. Even at this early stage in the analysis of the *bld* mutants, it is clear that the genes they identify play key roles in the complex process of cellular differentiation in *Streptomyces*.

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