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Critical Residues and Novel Effects of Overexpression of the *Streptomyces coelicolor* Developmental Protein BldB: Evidence for a Critical Interacting Partner▽†

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The *bldB* gene of *Streptomyces coelicolor* encodes the best-characterized member of a family of small proteins that have low isoelectric points but that lack any previously characterized sequence motifs. BldB is dimeric and is required for the efficient production of antibiotics and spore-forming cells, called aerial hyphae, by growing colonies. The mechanism of action of BldB and its relatives is unknown. Here, we have explored amino acids in BldB that either are highly conserved or have been implicated in function genetically. We show that five amino acids are important for its function at physiological expression levels. Mutations in three of these amino acids gave rise to proteins that were either monomeric or unstable in vivo, while two others are not. We find that overexpression of *bldB* in *S. coelicolor* blocks sporulation prior to sporulation-specific septation but permits the formation of aerial hyphae. Vegetative septation was apparently normal in both the *bldB* null mutant and the *bldB* overexpression strain. To our surprise, overexpression of the dimerization-competent but functionally defective alleles caused a dramatic acceleration of sporulation. Our results suggest that BldB makes at least one important contact with another subcellular constituent and that a loss or alteration of this interaction impairs the phenotypic properties of the organism.

The bacterium *Streptomyces coelicolor* is the best-characterized model organism for the spore-forming *Streptomyces* genus. These organisms are filamentous bacteria that are characterized by a robust secondary metabolism that has been exploited as a source of antibiotics, chemotherapeutic agents, antifungal drugs, immune suppressants, and other medicinally and agriculturally important molecules (3). The life cycle of this organism is especially complex for a bacterium. When spores germinate, they give rise to filamentous cells referred to as "substrate hyphae." These substrate hyphae grow by elongating and branching and form septal cross walls at infrequent intervals such that each cell is an elongated compartment housing multiple chromosomes. After ~48 h (under laboratory conditions), a second filamentous cell type appears on the colony surface and grows up into the air, forming a white layer referred to as an "aerial mycelium." Individual aerial hyphae adopt a coiled shape as they mature. Antibiotic production commences at around the same time as the formation of the aerial mycelium. In *S. coelicolor*, secondary metabolism is readily visible, as two of the antibiotics produced by this organism are pigmented: the polyketide actinorhodin is blue, and the tripyrrole undecylprodigiosin is red. Spore formation occurs exclusively in the aerial hyphae, and this too be can be readily visualized as a gray polyketide pigment is deposited in the maturing spore wall (12).

Mutations that interrupt this life cycle can be divided into several categories. The *bld* mutants block the formation of the aerial hyphae, giving rise to colonies that lack the fuzzy surface layer (13). Some *bld* mutations also block the production of antibiotics such that colonies lack the red and blue pigmentation that is characteristic of wild-type *S. coelicolor*. Mutations in the *whi* genes permit the propagation of the aerial mycelium but prevent the normal maturation of spores such that colonies fail to acquire their usual gray pigmentation and instead maintain a white aerial mycelium (5). Finally, genes that, when mutated, impair secondary metabolism but permit the formation of the aerial mycelium and the production of spores have been identified previously (see, e.g., reference 1).

The *bldB* gene encodes a small acidic protein (pI ~4.2) that has a monomeric molecular mass of 10.9 kDa and that is normally dimeric (7, 9, 21). Null mutations in *bldB* confer a profound defect in both the formation of aerial hyphae and the production of antibiotics (7, 17). Indeed, while the phenotypic defects of many *bld* mutations can be at least partially reversed by cultivation on media that have poor carbon sources, the defects conferred by *bldB* mutations cannot (22). Furthermore, there is evidence that strains bearing mutations in *bldB* are defective in normal carbon metabolism, such that genes that are normally repressed by the presence of glucose are expressed (22).

BldB lacks obvious sequence motifs of known function. It is, however, related to the products of at least 24 (Fig. 1a) other genes in the *S. coelicolor* genome. Included among these are the product of an open reading frame in the *abaA* locus (SCO0703), a protein that has been implicated in the production of several antibiotics, and the product of an open reading frame (SCO4542) lying immediately downstream of *whiJ*, which is important for spore formation by aerial hyphae. We

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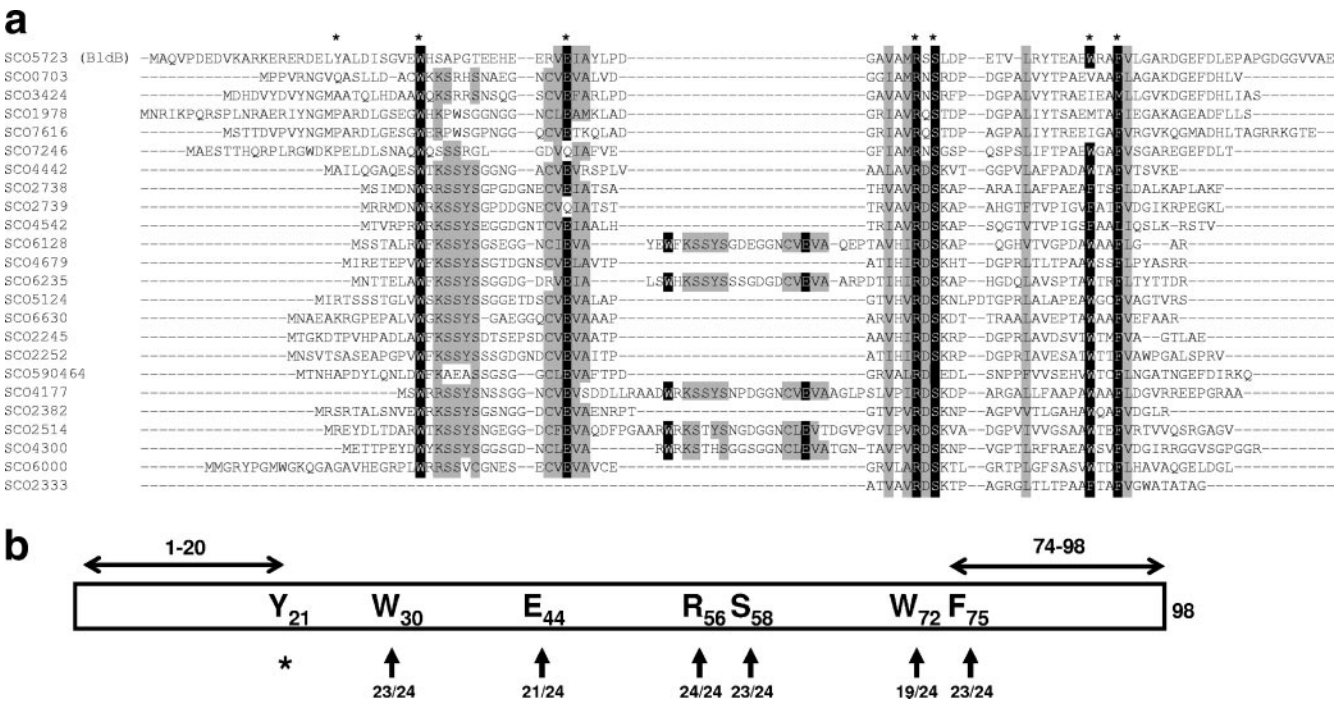

FIG. 1. Highly conserved residues in members of the BldB family. (a) Sequence alignment of BldB homologues in *Streptomyces coelicolor*. Homologues were identified using PSI-BLAST (NCBI) and are identified by the SCO numbers (2). Conserved residues are emphasized by gray (moderately conserved) or black (nearly universally conserved) shading. BldB residues that were mutated in this work are indicated by asterisks. An internal repeat within five of the protein sequences is evident. (b) Cartoon of BldB emphasizing residues 21, 30, 44, 56, 58, 72, and 75, which were mutated in this work, and the N- and C-terminal truncations that can be made without compromising dimer formation (7). The degree of conservation of these residues in the 24 BldB homologues of *S. coelicolor* is indicated.

have conducted an exhaustive search of DNA sequence databases and discovered that the *bldB*-like family of genes is restricted to filamentous actinomycetes. We have explored the role of six highly conserved amino acids in BldB and one amino acid that was previously implicated in function. Of these seven amino acids, we found that two are unimportant for function in vivo and that the other five are critical. Of these five amino acids, we show that two give rise to stable proteins that nevertheless fail to function at normal physiological levels. We find that the overexpression of *bldB* blocks sporulation in aerial hyphae but that the overexpression of the two dimerization-competent but defective alleles causes accelerated sporulation. These data suggest that BldB must have at least one interaction with another cellular constituent that is important for its biological function.

MATERIALS AND METHODS

Sequence alignment. The full-length BldB amino acid sequence was used as a query in a PSI-BLAST search at the NCBI website. Repeated iterations yielded a list of 89 homologues from which the *S. coelicolor* proteins were extracted. Alignments were performed using ClustalX (24) followed by manual adjustment. To attempt to rule out the existence of *bldB*-like genes in the *Mycobacteria* and *Corynebacteria*, we conducted more targeted BLAST searches of their genomes, none of which revealed any statistically relevant homologues.

Site-directed mutagenesis. Mutagenesis was carried out on plasmid pRA1 (the plasmids and strains used in this work are listed in Tables 1 and 2, respectively) at the amino acid residues indicated by boxes in Fig. 1 by using the oligonucle-

TABLE 1. Plasmids used in this study

Plasmid	Description[a]	Phenotype[b]	Source or reference
S. coelicolor			
pIJ486	*ter neo tsr ori* pIJ101 *rep* pIJ101 MCS	Thio^r	14
pIJ486BB	*ter neo tsr ori* pIJ101 *rep* pIJ101 MCS *bldB lacZ*α *aac(3)IV ori* pUC18	Apr^r/Thio^r	This work
pRA1	*lacZ*α *aac(3)IV ori* pUC18 *oriT* (RK2) *int* φC31 ϕC31 *attP* MCS *bldB* + 304-bp promoter region	Apr^r	7
E. coli			
pT18NHB	*bla ori colE1 f1* origin T18 MCS 0.32-kb KpnI amplicon from pBB801 containing *bldB* inserted into pT18	Amp^r	6
pT25NHB	*cat ori p15A T25* MCS 0.32-kb KpnI amplicon from pBB801 containing *bldB* inserted into pT25	Chl^r	7

[a] MCS, multiple cloning site.
[b] Antibiotic resistance markers are apramycin (Apr^r), ampicillin (Amp^r), thiostrepton (Thio^r), and chloramphenicol (Chl^r).

TABLE 2. Strains used in this study

Strain	Description[a]	Phenotype	Source or reference
S. coelicolor			
M145	Prototroph SCP1$^-$ SCP2$^-$		Lab collection
N985	*bldB*::*aphI* SCP1$^-$ SCP2$^-$	Bld	7
N17	*bldK*::Spec^r SCP1$^-$ SCP2$^-$	Bld	19
N376	*ramR*::Apr^r SCP1$^-$ SCP2$^-$	Bld	20
J2401	*whiA*::Hyg^r SCP1$^-$ SCP2$^-$	Whi	8
J2402	*whiB*::Hyg^r SCP1$^-$ SCP2$^-$	Whi	8
J2400	*whiG*::Hyg^r SCP1$^-$ SCP2$^-$	Whi	8
J2210	*whiH*::Hyg^r SCP1$^-$ SCP2$^-$	Whi	8
J2407	*sigF*::Tsr^r SCP1$^-$ SCP2$^-$	Whi	8
GSB1	*ssgB*::Apr^r SCP1$^-$ SCP2$^-$	Whi	11
E. coli			
Er^2-1	F′ *lacI*^{Q} *leuB6 thi-1 fhuA31 lacY1 tsx-78 galK2 galT22 supE44 hisG4 rpsL136* (Str^r) *xyl-5 mtl-1 dam13*::Tn*9* (Cam^r) *dcm-6 mcrB1 mcrA hsdR2* (r_K^- m_K^+)		16
XL-1 Blue	*recA1 endA1 gyrA96 thi-1 glnV44 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^r)]		Stratagene

[a] Antibiotic resistance markers are apramycin (Apr^r), ampicillin (Amp^r), hygromycin (Hyg^r), kanamycin (Kan^r), and spectinomycin (Spec^r).

otides listed in Table 3 and Quickchange mutagenesis (Stratagene). Each codon of interest was altered to code for an alanine residue. The same oligonucleotides were used to introduce the desired changes into the two-hybrid plasmids pT18NHB and pT25NHB (7). All mutations were confirmed by DNA sequencing.

Cloning of the BldB overexpression vector pIJ486BB. The high-copy-number *Streptomyces* vector pIJ486 was isolated from *Streptomyces lividans* and fused to a fragment of pRA1 that included an *Escherichia coli* origin of replication, the *bldB* gene and promoter, and an apramycin resistance gene to generate pIJ486BB. Plasmid pRA1 was digested with BamHI and NheI, liberating a DNA fragment that included the *bldB* gene, the apramycin resistance gene, and the pUC18 origin of replication. pIJ486 was cut with BamHI and HindIII to generate a fragment bearing the pIJ486 origin of replication, and the two fragments were ligated and transformed into *E. coli*. The same cloning procedure was repeated for all of the point mutants in pRA1, generating overexpression plasmids for wild-type *bldB* and for all of the mutant forms of the gene.

Electron microscopy. N985, a *bldB* null mutant (7) containing pIJ486, and wild-type strain M145 containing pIJ486, pIJ486BB, and the pIJ486BBF75A *bldB*(*F75A*) overexpression vector were cultivated on R2YE solid medium (14) for 48 h and prepared for electron microscopy.

Cells were fixed by the addition of a layer of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 30 min at room temperature. The samples were then rinsed in 0.2 M sodium cacodylate buffer (pH 7.4). Once rinsed, they were postfixed in 1% osmium tetraoxide in 0.1 M sodium cacodylate buffer (pH 7.4) for 30 min at room temperature. Upon completion of the fixation procedure, the samples were isolated and dehydrated in an ethanol series of 50, 70, 95, and 100% ethanol.

For transmission electron microscopy, samples that were first dehydrated with ethanol were further dehydrated with propylene oxide, infiltrated with Spurr's resin, and polymerized in 100% Spurr's resin at 60°C overnight. The samples were then cut into thin (70-nm) sections and stained with uranyl acetate and lead citrate. After staining, the samples were imaged using a JEOL 1200EX Toyoko transmission electron microscope.

For scanning electron microscopy, ethanol-dehydrated samples were critical point dried and gold coated to 20 to 40 nm. Once coated with gold, samples were viewed on the JEOL 840 Toyoko scanning electron microscope.

RESULTS AND DISCUSSION

We have conducted an extensive search for genes encoding proteins homologous to BldB using repeated iterations of PSI-BLAST. In total, there were 89 BldB-like proteins encoded in four bacterial genera. These included 24 clearly BldB-like gene products in the *S. coelicolor* genome (Fig. 1a), 25 in *Streptomyces avermitilis*, 5 in other streptomycetes whose genomes are only partly sequenced (2, 10), and 3 in other actinomycetes. There were 11 BldB-like proteins in the moderately thermophilic organism *Thermobifida fusca*, 7 in the human pathogen *Nocardia farcinica*, and 16 in the N_2-fixing endosymbiont *Frankia*. The unifying feature of this remarkably diverse group of organisms is that they are all filamentous (23). Three of

TABLE 3. Oligonucleotides used for mutagenesis in this study

Oligonucleotide	Sequence (5′–3′)	End use
Y21P1	CGAGCTGGCCGCGCTCGAC	Y21A mutagenesis
Y21P2	GTCGAGCGCGGCCAGCTCG	Y21A mutagenesis
W30P1	CTCGACATCTCGGGTGTGGAGGCGCACAGCGCG	W30A mutagenesis
W30P2	CGCGCTGTGCGCCTCCACACCCGAGATGTCGAG	W30A mutagenesis
E44P1	GGAACACGAGGAGCGGGTGGCGATCGCCTATC	E44A mutagenesis
E44P2	GATAGGCGATCGCCACCCGCTCCTCGTGTTCC	E44A mutagenesis
R56P1	CCGACGGAGCCGTGGCCATGGCGTCGTCGCTG	R56A mutagenesis
R56P2	CAGCGACGACGCCATGGCCACGGCTCCGTCGG	R56A mutagenesis
S58P1	GAGCCGTGGCCATGCGGTCGGCGCTGGATCC	S58A mutagenesis
S58P2	GGATCCAGCGCCGACCGCATGGCCACGGCTC	S58A mutagenesis
W72P1	CTGCGGTACACCGAGGCGGAGGCGCGGGCTTTC	W72A mutagenesis
W72P2	GAAAGCCCGCGCCTCCGCCTCGGTGTACCGCAG	W72A mutagenesis
F75P1	CCGAGGCGGAGTGGCGGGCTGCCGTCCTGGGTG	F75A mutagenesis
F75P2	CACCCAGGACGGCAGCCCGCCACTCCGCCTCGG	F75A mutagenesis

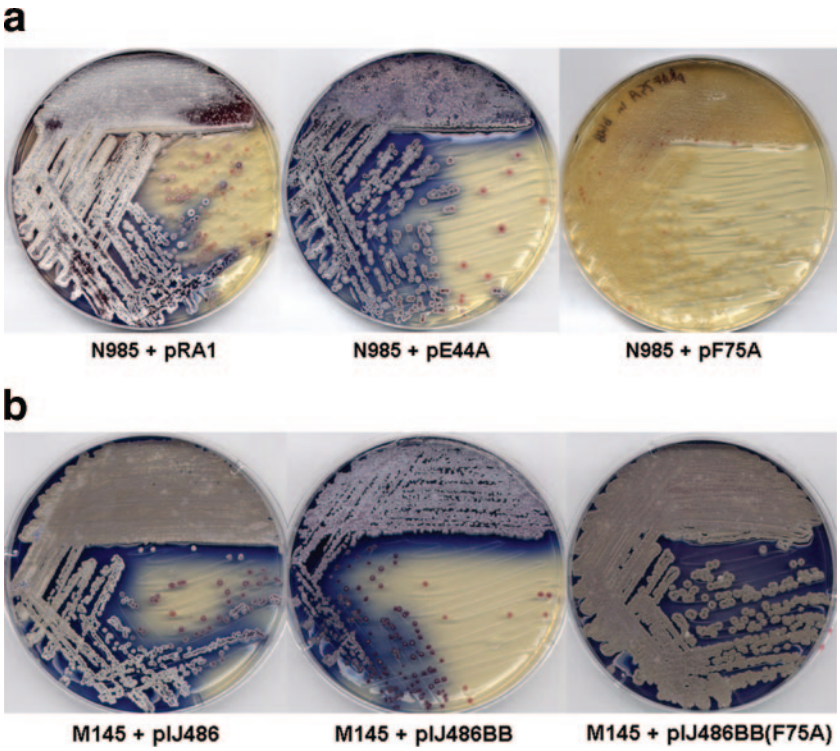

Fig. 2. Effect of *bldB* mutations and overexpression on *S. coelicolor*. (a) Complementation of the *bldB* null mutant (N985) with the wild-type, the E44A, and the F75A *bldB* alleles at low copy number (one to three copies/chromosome). The wild-type and E44A alleles have clearly restored the formation of aerial hyphae and the production of pigmented antibiotics. In contrast, the presence of the F75A allele had no effect on the mutant, which remained bald and unpigmented. (b) Effect of the same alleles at high copy number. Cells were grown for 48 h on R2YE medium.

them are also spore-forming bacteria, and while *N. farcinica* is not, its vegetative hypha cells undergo a morphological transition (fragmentation) that similarly results in dispersal and smaller units (23). Intriguingly, searches of the genomes of two other actinomycetes, *Mycobacteria* and *Corynebacteria*, did not reveal any genes encoding BldB-like proteins. In contrast to the other four, these actinomycetes are rod-shaped bacteria and do not produce spores, suggesting that the *bldB* family of genes may be restricted to filamentous actinomycetes. The significance of this is unknown but will surely be relevant to future investigation.

Alignment of the BldB-like protein sequences (see Fig. 1a for *S. coelicolor* and Fig. S1 in the supplemental material for the entire BldB-like family) revealed four blocks of relatively conserved sequence. In the *S. coelicolor* group, which includes representatives of all major features, 17 sequences exhibit the highly conserved WXK/RSSYS and CVEV/IA sequences (motifs I and II) near their amino termini. Five of these sequences have a second repeat of these motifs further into the protein (motifs I′ and II′ in Fig. 1a). BldB has a good match for motif II but not motif I, although it does have a W at residue 30, which is universally conserved in the family. The conserved sequences V/IA/HV/IRDSK (motif III) and AW/FXXFV/L (motif IV) were found progressively further towards the C termini, and BldB exhibits reasonable matches to both. We examined BldB in the context of these conserved blocks of sequence and identified six residues that were of particular interest (Fig. 1b). In addition to being highly conserved, these residues had hydrophilic side chains (E44, R56, and S58) or aromatic side chains (W30, W72, and F75), some of which might be surface exposed. We were less interested in conserved L, I, and V residues, assuming that these residues are more likely to be buried folding determinants. We targeted W30, E44, R56, S58, W72, and F75 for alanine substitution in the context of a previously constructed *bldB* complementation plasmid, pRA1 (7). We also constructed an alanine substitution at Y21, a residue previously demonstrated to be important for *bldB* function, as it is a frequent site of defective alleles of the gene (21). pRA1 is a low-copy-number vector that integrates into the *S. coelicolor* genome via a bacteriophage integrase mechanism (4). Between one and three copies of this vector integrate at a time (6).

We introduced each mutation into pRA1 and then inserted the resulting plasmids, designated pY21A through pW75A, into a *bldB* null mutant strain, N985, and its developmentally competent parent, M145, to assess the ability of each allele to drive aerial mycelium formation relative to the wild-type gene. At low copy numbers, none of these alleles had any effect on growth or development in M145 (data not shown); however, there were a number of important effects when the complementing alleles were introduced into the *bldB* null mutant (Fig. 2). As expected, pRA1 restored aerial mycelium formation to N985 such that development was normal (Fig. 2a, left plate). The mutations in amino acids E44 (Fig. 2a, middle plate) and S58 (data not shown) had little or no effect on gene function: both supported the formation of an aerial mycelium at levels

TABLE 4. Effects of *bldB* mutations on function in vivo[a]

Allele	Dimerization	Single copy	Overexpression
Wild type	+	+	Whi
Y21A	+	−	Rsp
W30A	−	−	—
E44A	+	+	NT
R56A	−	−	—
S58A	+	+	NT
W72A	−	−	—
F75A	+	−	Rsp

[a] The capacity or inability of each allele to dimerize or complement in single copy is indicated by a "+" or "−," respectively. The phenotype conferred by each overexpressed allele is indicated by "—" (no effect), NT (not tested), Whi (white), or Rsp (rapid sporulation).

similar to those of the wild-type gene, suggesting that these residues are relatively unimportant for function in vivo. In contrast, mutations that changed R56, Y21, W30, W72, and F75 (Fig. 2a, right plate) did not restore antibiotic-associated pigmentation or the formation of aerial hyphae to the mutant, suggesting that these residues are important for BldB function.

We have previously used two-hybrid analysis to demonstrate that BldB (7) and one of its homologues, the product of the gene SCO0703 in the *abaA* locus (M. Eccleston and J. R. Nodwell, unpublished observations), form homodimers. To determine whether these mutations had any effect on dimer formation, we introduced each sequence change into the bait and target plasmids that we constructed previously and assessed the ability of each mutant to dimerize in *E. coli*. Consistent with the fact that both alleles functioned normally in morphogenesis, BldB(E44A) and BldB(S58A) were both able to dimerize. In contrast, three of the defective alleles, W30, R56, and W72 exhibited defects in dimer formation, suggesting that these residues may form part of the dimer interface. We did not confirm, however, that these three alleles gave rise to stable proteins in vivo, so it is also possible that they simply encode proteins that fail to fold correctly. More importantly, two of the defective alleles, Y21A and F75A, encoded proteins that were able to homodimerize in vivo. The two-hybrid system that we used for this analysis is not quantitative; however, the behavior of these strains was indistinguishable from the behavior of those strains encoding the wild-type alleles of BldB. These data suggest that Y21 and F75 are important for BldB function but dispensable for dimer formation. The properties of all *bldB* alleles, including those described below, are summarized in Table 4.

In parallel with this work, we assessed the effects of placing *bldB*, under the control of its own promoter, onto a high-copy-number vector resulting in the overexpression in *S. coelicolor* (Fig. 2b). The high-copy-number vector lacking *bldB* had no effect on the growth or development of M145 (Fig. 2b, left plate). To our surprise, we found that when the wild-type allele was present at high copy numbers in wild-type *S. coelicolor*, colonies exhibited a phenotype reminiscent of that of the *whi* mutants, suggesting a defect in sporulation (Fig. 2b, middle plate). The aerial mycelium formed under these conditions was somewhat sparse compared to that of phenotypically wild-type strains, but it was reproducible. Antibiotic-associated pigmentation appeared normal. We observed a similar defect when we introduced the *bldB* overexpression plasmid into *bldB* null mutant strain N985 and into another morphologically wild-type strain, M600 (data not shown). While development did not proceed any further in any of the three strains with continued incubation, M145 containing the control plasmid sporulated to completion within 4 days.

To determine the point in development where a high copy number of *bldB* induced arrest, we conducted scanning electron microscopy (Fig. 3). The surface of M145 colonies bearing a control plasmid and grown for 48 h had clearly initiated the formation of aerial hyphae, as demonstrated by the presence of coiled filaments and some mature spores. In contrast, M145 overexpressing *bldB* was blocked prior to sporulation septation: all hyphae were smooth and lacked the regularly spaced perforations of septating hyphae and there were no coiled filaments or mature spores. We also noticed a tendency for these aerial hyphae to be clumped in some places. Cells on the surface of *bldB* null mutant colonies did not exhibit any of the properties of aerial hyphae. We also conducted transmission electron microscopy on these strains to determine whether they were able to carry out vegetative septation (Fig. 3b). Vegetative septa were detectable in both the wild-type strain containing a high copy number of *bldB* and in the *bldB* null mutant. The developmental block caused by the overexpression of wild-type *bldB* therefore appears to be later than the erection of the aerial mycelium but earlier than the sporulation septation step.

To determine the effects of *bldB* mutations on this dominant effect, we constructed identical overexpression vectors for each of the five defective alleles listed in Table 4 and introduced these vectors into M145. The *bldB*(*W30A*), *bldB*(*R56A*), and *bldB*(*W72A*) alleles had no effect on morphogenesis, consistent with the fact that their products were otherwise devoid of activity. The fact that the increased copy number of these alleles had no effect on development demonstrated that the phenotypic effects of overexpression of the wild-type gene were due to the BldB protein and not due to the titration of transcription factors by *bldB* promoter DNA.

In marked contrast, overexpression of the *bldB*(*Y21A*) and *bldB*(*F75A*) alleles caused a dramatic acceleration of morphogenesis such that within 48 h of growth, the colonies were completely covered in a mature, deep gray aerial mycelium (Fig. 2a, right plate). When viewed under the scanning electron microscope (Fig. 3a), the surfaces of these strains were almost completely covered with mature spores, and development was clearly accelerated relative to the control-plasmid-containing strain.

These dominant effects of *bldB* overexpression on morphogenesis by wild-type colonies suggested that while excess levels of the wild-type protein inhibited sporulation, similar levels of dimerization-competent, morphogenesis-defective proteins caused it to proceed in an accelerated manner, reminiscent of the "rapid aerial mycelium" or Ram phenotype conferred by overexpression of the *ram* genes (15, 18). We introduced the overexpression plasmids for the Y21A and F75A mutants into strains bearing null mutations in the genes *bldK*, *ramR*, *whiA*, *whiB*, *whiG*, *whiH*, *sigF*, and *ssgB*, all of which interrupt development at various stages. Neither overexpression plasmid caused a bypass of the effects of any of the mutations. This suggests that the accelerated spore formation brought about by

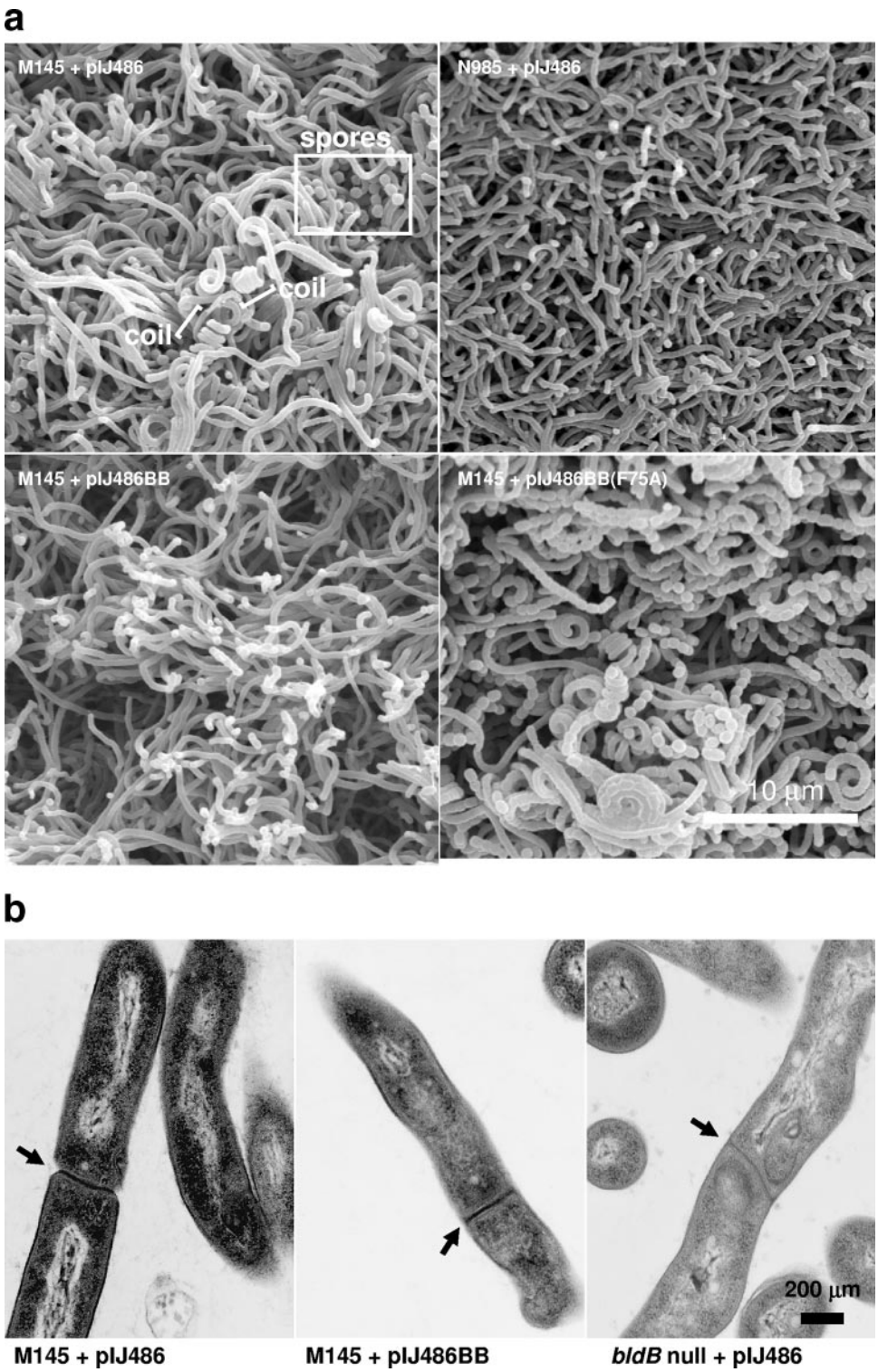

FIG. 3. Electron microscopy of phenotypes conferred by *bldB* overexpression. (a) Scanning electron microscopy of the surfaces of colonies of wild-type strain M145 containing a high-copy-number control plasmid (pIJ486), the *bldB* overexpression plasmid (pIJ486BB), and the *bldB*(*F75A*) plasmid [pIJ486BB(F75A)]. As an additional control, the *bldB* null mutant N985 containing the high-copy-number control plasmid (pIJ486) is also shown. Examples of coiled aerial filaments and mature spores are indicated. (b) Transmission electron microscopy of M145 containing a high-copy-number control plasmid, M145 containing the *bldB* overexpression plasmid, and the *bldB* null mutant N985 containing the high-copy-number control plasmid. Septa are indicated by arrows.

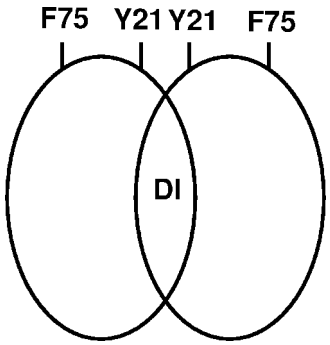

FIG. 4. BldB is a dimeric protein with at least one interaction surface. The shared phenotypes conferred by mutations in Y21 and F75 suggest that these residues fulfill similar functions. We suggest that this is an interaction and that these residues are part of the same surface. BldB must also have a dimerization interface (DI), and it is possible that one or more of the highly conserved residues W30, R56, or W72 make up part of this surface.

the overexpression of *bldB*(*Y21A*) or *bldB*(*F75A*) is dependent on the normal genetic machinery involved in morphogenesis.

The fact that the Y21A and F75A mutations confer identical phenotypes under a variety of expression conditions suggests to us that these residues fulfill related roles for the protein. An obvious explanation is that they are located close to each other in the three-dimensional structure of the protein (Fig. 4) and that they constitute part of an interaction surface for the binding of another molecule, most likely another protein. Altering either residue might therefore compromise or alter this interaction, bringing about dramatic changes in the sporulation properties of the organism. F75 is located in the most C terminal of the conserved blocks of sequence in the BldB family (Fig. 1a). Indeed, this residue is found in almost all family members (see Fig. S1 in the supplemental material), suggesting perhaps that many or most of these proteins make similar use of this motif. Identifying this BldB partner protein promises to provide major insights into sporulation in *S. coelicolor* and into the mechanism of action of the BldB family of proteins.

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