The actinobacteria-specific gene *wblA* controls major developmental transitions in *Streptomyces coelicolor* A3(2)

Kay Fowler-Goldsworthy,¹ Bertolt Gust,^{1†} Sébastien Mouz,^{1‡}
Govind Chandra,¹ Kim C. Findlay² and Keith F. Chater¹

Correspondence

Keith F. Chater

keith.chater@bbsrc.ac.uk

¹Department of Molecular Microbiology, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

²Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

The *Streptomyces coelicolor* A3(2) sporulation gene *whiB* is the paradigm of a family of genes (*wbl*, *whiB*-like) that are confined to actinobacteria. The chromosome of *S. coelicolor* contains 11 *wbl* genes, among which five are conserved in many actinobacteria: *whiB* itself; *whiD*, a sporulation gene; *wblC*, which is required for multi-drug resistance; and *wblA* and *wblE*, whose roles had previously been little studied. We succeeded in disrupting *wblA* and the six non-conserved genes, but could not disrupt *wblE*. Although mutations in the six non-conserved *wbl* genes (including some multiple *wbl* mutants) produced no readily detectable phenotype, mutations in *wblA* had novel and complex effects. The aerial mycelium of *wblA* mutants was coloured red, because of the ectopic presence of pigmented antibiotics (actinorhodin and undecylprodigiosin) normally confined to lower parts of wild-type colonies, and consisted almost entirely of non-sporulating, thin, straight filaments, often bundled together in a fibrillar matrix. Rare spore chains were also formed, which exhibited wild-type properties but were genetically still *wblA* mutants. A *wblA* mutant achieved higher biomass than the wild-type. Microarray analysis indicated major transcriptional changes in a *wblA* mutant: using a relatively stringent cut-off, 183 genes were overexpressed, including genes for assimilative primary metabolism and actinorhodin biosynthesis, and 103 were underexpressed, including genes associated with stages of aerial hyphal growth. We suggest that *WblA* is important in both the slow-down of biomass accumulation and the change from aerial hyphal initial cells to the subapical stem and apical compartments that precede sporulation; and that the mutant aerial mycelium consists of recapitulated defective aerial hyphal initial cells.

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INTRODUCTION

Gram-positive bacteria with high G+C content in their DNA (the phylum Actinobacteria) are abundant and ubiquitous. Ventura *et al.* (2007) carried out a comparative analysis of more than 20 sequenced genomes of actinobacteria, including major pathogens (e.g. the agents of tuberculosis and diphtheria), industrially important producers of amino acids (e.g. *Corynebacterium glutamicum*), agents of

bioremediation (e.g. *Rhodococcus* spp.), members of the important symbiotic nitrogen-fixing genus *Frankia*, and streptomycetes, the subject of this paper. Streptomycetes play a major role in soil ecology, are the most important source of antibiotics and other secondary metabolites, and exhibit unusual morphological complexity.

Some gene families are both peculiar to, and universal among, free-living actinobacteria (Gao *et al.*, 2006; Ventura *et al.*, 2007). Here we focus on one such family of paralogues, whose first identified member, *whiB*, was discovered by analysis of DNA that complemented a *Streptomyces coelicolor* A3(2) mutant defective in the formation of spores on aerial hyphae (Davis & Chater, 1992). Another sporulation gene, *whiD*, is also *whiB*-like (Molle *et al.*, 2000). As it became clear that streptomycetes and other actinomycetes possessed multiple *whiB*-like genes, the general designation '*wbl*' was suggested for the

[†]Present address: Pharmaceutical Institute, University of Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany.

[‡]Present address: Solid Drug Development SA, 8 Road John Grasset, 1205 Geneva, Switzerland.

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gene family (Soliveri *et al.*, 1993, 2000), although there are various designations in other organisms (*whiB1-7* in *Mycobacterium tuberculosis* and *Mycobacterium leprae*; *whmA-E* in *Mycobacterium smegmatis*; and *whc* in *Corynebacterium glutamicum*). In morphologically simple mycobacteria, the *whiB* orthologue is required for proper cell division (Gomez & Bishai, 2000; Raghunand & Bishai, 2006a, b) and the *whiD* orthologue for survival in the *M. tuberculosis*-infected host (Steyn *et al.*, 2002), while the seven *wbl* genes of *M. tuberculosis* all show different responses to various imposed stresses (Geiman *et al.*, 2006), *wblC* mutants (in mycobacteria and *Streptomyces lividans*) notably having increased sensitivity to a wide range of antibiotics (Morris *et al.*, 2005). A *wblE* mutant of *S. coelicolor* had no obvious phenotype (Homerová *et al.*, 2003), although disruption of an apparently orthologous gene of *C. glutamicum* caused increased sensitivity to oxidative stress (Kim *et al.*, 2005).

Wbl proteins contain four conserved cysteine residues, which are important for function and coordinate a redox-sensitive iron-sulphur cluster (Jakimowicz *et al.*, 2005; Alam *et al.*, 2007, 2009; Singh *et al.*, 2007; Raghunand & Bishai, 2006a; Crack *et al.*, 2009; Rybniker *et al.*, 2010). Because of this, Wbl proteins were reviewed in the general context of redox regulation in actinobacteria by den Hengst & Buttner (2008), who found more than 270 *wbl* genes in the sequence database. Non-specific protein disulphide reductase activity was associated with most of the WhiB-like proteins of *M. tuberculosis* by one laboratory (Garg *et al.*, 2007; Alam *et al.*, 2007, 2009), although others were unable to detect such activity with WhiB1 or the *S. coelicolor* orthologue of WhiB3 (Crack *et al.*, 2009; Smith *et al.*, 2010).

In mycobacteria, direct experimental evidence of DNA-binding regulatory roles has been obtained for WhiB3 (Guo *et al.*, 2009; Singh *et al.*, 2009), for WhiB1 (Smith *et al.*, 2010), and for WhiB2 and a mycobacterial phage-encoded example (Rybniker *et al.*, 2010). Strikingly, binding of WhiB1 to a specific DNA target, resulting in repression of transcription *in vitro*, required interaction of the iron-sulphur cluster with nitric oxide (NO), an interaction that is specific and very rapid (Smith *et al.*, 2010): WhiD of *S. coelicolor* also interacts strongly with NO (Crack *et al.*, 2011). This suggests that Wbl proteins in general may change their regulatory properties in response to NO generated either exogenously (e.g. by hosts responding to pathogens) or endogenously by bacterial NO synthase, for example during stress responses (Smith *et al.*, 2010; Gusarov *et al.*, 2009). An earlier study reported that WhiB3 interacts with the principal sigma factor (Steyn *et al.*, 2002), and several *wbl* genes of *S. coelicolor* lie very close to genes for sigma factors (Chater & Chandra, 2006) – indeed, one plasmid-linked gene, *wblP*, encodes a predicted Wbl-sigma fusion protein (Bentley *et al.*, 2004).

In addition to *whiB* and *whiD*, the *S. coelicolor* chromosome (Bentley *et al.*, 2002) contains nine other *wbl*

genes, which are surveyed by gene disruption in this paper. It emerged that *wblA*, one of the five widely conserved *wbl* genes, is important in determining whether emerging aerial hyphae will go on to sporulate, as well as in initiating other physiological changes that follow the main period of biomass accumulation. This complements independent evidence that multiple copies of *wblA* repress antibiotic production (Kang *et al.*, 2007).

METHODS

Strains, plasmids and conditions for growth and targeted mutagenesis. Strains and plasmids used in this study are summarized in Table 1. *Streptomyces* media and culture conditions were as described by Kieser *et al.* (2000). *S. coelicolor* strains were grown on agar media MS (soya flour with mannitol), MM (minimal medium, containing 0.5% mannitol as carbon source unless otherwise specified) or R2YE (hypertonic, containing yeast extract). For DNA extraction, *Streptomyces* strains were incubated in YEME liquid medium containing glycine (0.5%). Transformation of *Escherichia coli* by electroporation, and general molecular genetics techniques, were as in Sambrook & Russell (2001). *E. coli* DH5 α was used for standard propagation of plasmids, and BW25113/pIJ790 (which contains the arabinose-inducible λ -RED system) was used for PCR-targeted mutagenesis (Datsenko & Wanner, 2000) as modified for *Streptomyces* (Gust *et al.*, 2003, 2004, 2006). The non-methylating *E. coli* strain ET12567, sometimes containing pUZ8002, was used to propagate unmethylated plasmids or cosmids prior to their introduction into *S. coelicolor*, bypassing its methyl-sensing restriction system. The cosmids used for targeted mutagenesis were H17 (*wblA*), 3B6 (*wblC*), 7G11 (*wblE*), 4C6 (*wblH*), K7 (*wblI*), 4B10 (*wblJ*), 5F8 (*wblK*), 6F7 (*wblL*) and 1B2 (*wblM*) (Redenbach *et al.*, 1996). The gene-specific extensions of PCR primers (see Supplementary Table S1, available with the online version of this paper) were designed to eliminate the whole of each target gene except the translational start and stop codons. After conjugation of mutated cosmids into *S. coelicolor*, kanamycin-sensitive (Kan^S), apramycin-resistant (Apra^R) double-crossover mutants with target genes replaced by the *aac(3)IV-oriT* cassette were confirmed by PCR amplification and Southern blot hybridization. In some cases, the marked disrupted gene in *S. coelicolor* was replaced by an unmarked version of the deleted gene, taking advantage of the FLP-mediated excision of the disruption cassette via FRT sites (FLP recognition targets) flanking the disruption cassettes (Gust *et al.*, 2006). For the construction of multiple *wbl* mutants, the *wblK* scar mutant J3913 was the starting point. Mutated versions of *wblE*, *wblH*, *wblI*, *wblJ*, *wblK*, *wblL* and *wblM* (all disrupted with *aac(3)IV-oriT*) in the respective cosmids were introduced into J3913 to generate double mutants, then scar-containing versions of each of the four genes were used to replace the Apra^R-marked versions to create Apra^S double mutants. These were then converted into various triple mutants by the introduction of the Apra^R-marked version of the appropriate gene.

Complementation of the *wblA* mutant. In one approach, the entire cosmid H17 was introduced into *wblA* mutants by conjugation from *E. coli* ET12567/pUZ8002, in the form of derivatives H17-787 (allowing site-specific integration at the ϕ C31 *attB* site) or H17-784 (which could integrate at the *wblA* region by homologous recombination) (Table 1). In the second approach, *wblA* was amplified by PCR, using 5'-phosphorylated primers. The PCR product was ligated with the integrative vector pSET152 or its *hyg*-containing derivative pIJ82 (each *EcoRV*-digested, and dephosphorylated by alkaline phosphatase). The ligation mixture was introduced into *E. coli* DH5 α to generate pSET152::*wblA* or pIJ82::*wblA* (with a

Table 1. Strains and plasmidsCosmids used for the disruption of *wbl* genes are listed in Methods.

Strain	Relevant features		Source or reference
J1916	$\Delta glkA$	J1501 derivative	Homerová <i>et al.</i> (2008)
J3900	<i>wblA::aac(3)IV</i>	M145 <i>wblA::aac(3)IV</i>	This paper
J3901	<i>redD wblA</i>	M510 <i>wblA::aac(3)IV</i>	This paper
J3902	<i>actII-orf4 wblA</i>	M511 <i>wblA::aac(3)IV</i>	This paper
J3903	<i>redD actII-orf4 wblA</i>	M512 <i>wblA::aac(3)IV</i>	This paper
J3904	<i>wblA::aac(3)IV/wblA⁺</i>	J3900::H17-787	This paper
J3905	<i>wblA::aac(3)IV/vector only</i>	J3900::Supercos 1-787	This paper
J3906	<i>wblA::aac(3)IV/wblA⁺</i>	J3900::H17-784	This paper
J3907	<i>wblA</i>	M145 <i>wblA::scar</i>	This paper
J3908	<i>wblA/wblA⁺</i>	J3907/pSET152:: <i>wblA</i>	This paper
J3909	<i>wblA</i>	J3907/pSET152	This paper
J3910	<i>wblA/wblA⁺</i>	M145/pSET152:: <i>wblA</i>	This paper
J3911	<i>wblK::aac(3)IV</i>	M145 <i>wblK::aac(3)IV</i>	This paper
J3913	<i>wblK</i>	J3911 <i>wblK::scar</i>	This paper
J3917	<i>wblM::aac(3)IV</i>	M145 <i>wblM::aac(3)IV</i>	This paper
J3920	<i>wblK wblI::aac(3)IV</i>	J3913 <i>wblI::aac(3)IV</i>	This paper
J3923	<i>wblK wblI</i>	J3920 <i>wblI::scar</i>	This paper
J3925	<i>wblK wblJ::aac(3)IV</i>	J3913 <i>wblJ::aac(3)IV</i>	This paper
J3929	<i>wblK wblJ</i>	J3925 <i>wblJ::scar</i>	This paper
J3932	<i>wblK wblL::aac(3)IV</i>	J3913 <i>wblL::aac(3)IV</i>	This paper
J3933	<i>wblK wblL</i>	J3932 <i>wblL::scar</i>	This paper
J3936	<i>wblK wblM::aac(3)IV</i>	J3913 <i>wblL::aac(3)IV</i>	This paper
J3940	<i>wblK wblI wblH::aac(3)IV</i>	J3923 <i>wblH::aac(3)IV</i>	This paper
J3943	<i>wblK wblI wblJ::aac(3)IV</i>	J3923 <i>wblJ::aac(3)IV</i>	This paper
J3946	<i>wblK wblI wblM::aac(3)IV</i>	J3923 <i>wblM::aac(3)IV</i>	This paper
J3948	<i>wblK wblJ wblH::aac(3)IV</i>	J3929 <i>wblH::aac(3)IV</i>	This paper
J3951	<i>wblK wblJ wblM::aac(3)IV</i>	J3929 <i>wblM::aac(3)IV</i>	This paper
J3954	<i>wblK wblL wblH::aac(3)IV</i>	J3933 <i>wblH::aac(3)IV</i>	This paper
J3957	<i>wblK wblL wblI::aac(3)IV</i>	J3933 <i>wblI::aac(3)IV</i>	This paper
J3960	<i>wblK wblL wblJ::aac(3)IV</i>	J3933 <i>wblJ::aac(3)IV</i>	This paper
J3963	<i>wblK wblL wblM::aac(3)IV</i>	J3933 <i>wblM::aac(3)IV</i>	This paper
J3971	<i>wblH::aac(3)IV</i>	M145 <i>wblH::aac(3)IV</i>	This paper
J3972	<i>wblH</i>	M145 <i>wblH::scar</i>	This paper
J3973	<i>wblI::aac(3)IV</i>	M145 <i>wblI::aac(3)IV</i>	This paper
J3974	<i>wblI</i>	M145 <i>wblI::scar</i>	This paper
J3975	<i>wblJ::aac(3)IV</i>	M145 <i>wblJ::aac(3)IV</i>	This paper
J3976	<i>wblJ</i>	M145 <i>wblJ::scar</i>	This paper
J3977	<i>wblL::aac(3)IV</i>	M145 <i>wblL::aac(3)IV</i>	This paper
J3978	<i>wblL</i>	M145 <i>wblL::scar</i>	This paper
M145, M600	Plasmid-free prototrophs		Kieser <i>et al.</i> (2000)
M510	<i>redD</i>	M145 $\Delta redD$	Florian & Bibb (1996)
M511	<i>actII-orf4</i>	M145 $\Delta actII-orf4$	Florian & Bibb (1996)
M512	<i>redD actII-orf4</i>	M145 $\Delta redD \Delta actII-orf4$	Florian & Bibb (1996)
Plasmids	Relevant features		Source or reference
Supercos 1	Cosmid vector, has ampicillin resistance gene for selection in <i>E. coli</i> and neomycin/kanamycin resistance gene for selection in <i>E. coli</i> and <i>Streptomyces</i>		Stratagene
Supercos 1-787	Supercos 1 derivative with the <i>bla</i> gene of the cosmid vector portion replaced by a segment from pIJ787 containing the <i>tet</i> resistance determinant for selection in <i>E. coli</i> , the <i>oriT</i> region of RK2 to permit mobilization from <i>E. coli</i> into <i>Streptomyces</i> , and the phiC31 <i>att</i> , <i>int</i> region for site-specific integration into <i>Streptomyces</i>		Gust <i>et al.</i> (2004)
H17	Large insert of <i>S. coelicolor</i> DNA including <i>wblA</i> cloned into Supercos 1		Redenbach <i>et al.</i> (1996)
H17-773	H17 derivative with <i>wblA</i> replaced by the apramycin-resistance gene of pIJ773		This work
H17 <i>wblA::scar</i>	H17-773 derivative with the 773 insert excised by FLP recombinase to leave an unmarked in-frame deletion		This work

Table 1. cont.

Strain	Relevant features	Source or reference
H17-784	H17 derivative in which the <i>bla</i> gene of the cosmid vector portion was replaced by the <i>aac(3)IV-oriT</i> segment from pIJ784	This work
H17-787	H17 derivative with the <i>bla</i> gene of the cosmid vector portion replaced by a segment from pIJ787 containing the <i>tet</i> resistance determinant for selection in <i>E. coli</i> , the <i>oriT</i> region of RK2 to permit mobilization from <i>E. coli</i> into <i>Streptomyces</i> , and the <i>phiC31 att</i> , <i>int</i> region for site-specific integration into <i>Streptomyces</i>	This work
pIJ82	Version of pSET152 containing <i>hyg</i>	H. M. Kieser, unpublished
pIJ82:: <i>wblA</i>	pIJ82 with <i>wblA</i> -specific fragment extending from 81 bp upstream of <i>wblA</i> to 55 bp downstream inserted at <i>EcoRV</i> site	This work
pIJ773*	Template for amplification of the apramycin-resistance cassette used for gene disruption	Gust <i>et al.</i> (2003, 2006)
pIJ784*	Template for amplification of the <i>oriT-apra</i> cassette used to replace <i>bla</i> in Supercos 1 derivatives	Gust <i>et al.</i> (2004)
pIJ787*	Template for amplification of the <i>oriT-tet-attP-int</i> cassette used to replace <i>bla</i> in Supercos 1 derivatives	Gust <i>et al.</i> (2004)
pIJ790*	λ -Red recombination plasmid	Gust <i>et al.</i> (2003)
pSET152	<i>E. coli</i> / <i>Streptomyces</i> vector plasmid mobilizable into <i>Streptomyces</i> , with <i>aac(3)IV</i> for selection and the ϕ C31 <i>int-att</i> system for site-specific integration into the <i>Streptomyces</i> chromosome	Bierman <i>et al.</i> (1992)
pSET152:: <i>wblA</i>	pSET152 with <i>wblA</i> -specific fragment extending from 460 bp upstream of <i>wblA</i> to 55 bp downstream, inserted at <i>EcoRV</i> site	This work
pUZ8002	RP4 <i>neo</i> , supplies <i>trans</i> -acting transfer functions for <i>E. coli</i> – <i>Streptomyces</i> conjugations	Paget <i>et al.</i> (1999)

*<http://streptomyces.org.uk/redirect/cassettes/index.html>.

shorter *wblA*-containing insert). The constructs were then mobilized (via ET12567/pUZ8002) into *S. coelicolor wblA* mutants. Exconjugants were checked by PCR before phenotypic testing.

Microscopy. Phase-contrast microscopy, scanning electron microscopy (SEM) and transmission microscopy of thin sections (TEM) were as described by Flårdh *et al.* (1999), Di Berardo *et al.* (2008) and Molle *et al.* (2000), respectively.

Resistance of spores to heat treatment. Spore suspensions in 20 % (v/v) glycerol were heated in a waterbath at 50 °C. Samples were removed at consecutive time points to ice before dilution and plating for viable counts (Molle *et al.*, 2000).

RNA isolation and S1 nuclease mapping. S1 nuclease mapping was as described by Jakimowicz *et al.* (2006), using the same RNA preparations and molecular mass markers as were used in Figure 5 of that paper. The probe was prepared by PCR amplification, using unlabelled oligo A1.wblA, and oligo A2.wblA labelled with ³²P. For RNA isolation prior to microarray analysis, *S. coelicolor* strains were germinated in 2 × YT for 6 h, and 3 × 10⁶ germinated spores were inoculated on cellophane discs on MM containing mannitol. The cultures were scraped off at 24, 36, 48, 60, 72 and 84 h (three replicate experiments were used for microarray analysis), then ground in liquid nitrogen, transferred to 50 ml Corning tubes, and suspended in 10 ml modified Kirby mix [1 g *N*-lauroylsarcosine (Sigma, L-9150), 6 g *p*-aminosalicylic acid sodium salt (Sigma, A-3505), 5 ml 2 M Tris/HCl pH 8, 6 ml buffered phenol pH 8, distilled H₂O to 100 ml]. Silica (Sigma, S9887) was added. After vortexing, sonication and several phenol/chloroform and chloroform extractions, the extracted RNA was treated with RNase-free DNase I, then the extractions with phenol/chloroform and chloroform were repeated. RNA was quantified by the Nanodrop spectrophotometer, and its quality assessed using the Agilent Bioanalyser. Genomic DNA contamination

was assayed by PCR using gene-specific primers. Samples with significant contamination (all samples of biological replicate 1) were digested with DNase I on RNeasy mini columns (Qiagen, 74104). Full details of the procedures have been deposited in Gene Expression Omnibus (see below).

Microarray hybridization. The synthesis and labelling of cDNA and genomic DNA (gDNA) and the array hybridization procedures were carried out according to the Microarray Hybridization Protocol of Mersinias and others (<http://www2.surrey.ac.uk/fhms/microarrays/>). Cy3-labelled cDNA and Cy5-labelled M145 gDNA were mixed before hybridization. Each slide hybridization used 12.5 µg cDNA and 3.5 µg sonicated gDNA. Each of the 36 cDNA/gDNA mixtures was hybridized to two microarrays (technical duplicates). Oligonucleotide microarray chips representing 7726 *S. coelicolor* genes and 278 SCP1 genes, with 1776 positive and negative control spots, were designed as in Bucca *et al.* (2003). The layout followed that of SCo3, and the slides were from print runs SCo6 and SCo7 (http://www2.surrey.ac.uk/fhms/microarrays/Downloads/grid_maps/).

Array data analysis. After hybridization, array chips were scanned (GenePix 4000B scanner) and the images quantified using GenePix Pro 5.0 (Axon Instruments) to generate the data files. The multi-image .tif files were quantified using BlueFuse, and the outputs saved as .xls files. These were read into the limma package of the R platform and then processed as described in the documentation of the limma package. Briefly, the function *loess* was used to normalize within arrays while scaling was used to normalize between arrays. Contrasts were defined as expression in *wblA* (mutant) minus expression in M145 (wild-type), and coefficients recalculated in terms of contrasts using the function *contrasts.fit*. Finally the function *eBayes* was used to compute (and rank genes by) the log-odds of differential expression. For the purpose of this paper, genes were considered to be influenced by the deletion of *wblA* if

the adjusted *P*-value of differential expression over all time points (calculated by the function *topTable*) was less than $1e^{-2}$ and there was a change in expression of at least twofold at one or more time points. In an initial basic authentication of the microarray analysis, mRNA of *wblA* itself had reduced representation in the mutant at all time points ($P=3.87e^{-8}$), as expected since *wblA* was entirely deleted from the mutant. The experimental details and data from the microarrays have been deposited as series accession number GSE24396 in Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24396>).

RESULTS

Systematic disruption of previously unstudied *wbl* genes of *S. coelicolor*

Screening by BLASTP for genes related to *whiB* revealed 11 *wbl* genes on the linear chromosome of *S. coelicolor* M145 (Bentley *et al.*, 2002). One of them, *wblL*, had not been recognized as a coding sequence in the original genome annotation, but has now been incorporated into the StrepDB database as SCO6965a (<http://streptomyces.org.uk>). The equivalence of the *S. coelicolor* *wbl* genes to *wbl* genes in other actinobacteria is shown in Table 2. Five (*wblA*, *whiB*, *wblC*, *whiD*, *wblE*) are present in most sequenced actinobacterial genomes, including the sequenced *Streptomyces* genomes (based on the genomes surveyed by Ventura *et al.*, 2007). The other six, which we

refer to as supernumerary, appear to occur only in streptomycetes: *wblH* and *wblI* in all of them, with the remainder (*wblJ*, *wblK*, *wblL*, *wblM*) occurring more sporadically. Most of the deduced Wbl proteins are small (81–125 aa), although a few are larger; and there is considerable diversity in their predicted pI values (Table 2).

To investigate the functions of the *wbl* genes, we used PCR targeting of segments of *S. coelicolor* DNA cloned in cosmids to construct *aac(3)IV*-marked deletion-replacement *wbl* mutations lacking all but the start and stop codon. The mutations were introduced into *S. coelicolor* M145 by gene replacement. We did not include the previously studied sporulation genes *whiB* and *whiD* in this analysis. We succeeded in replacing eight of the nine genes, but failed with the widely conserved *wblE*, even though single-crossover integration of the mutated *wblE*-containing cosmid H17 was readily obtained. *wblE* is 3968 bp from one end of the H17 insert, so this relatively short interval might have been the cause of our failure to obtain the second crossover needed for gene replacement. However, this was unlikely, because we could readily replace SCO5239, located between *wblE* (SCO5240) and the nearer H17 cosmid end, in a control experiment. It is therefore possible that elimination of *wblE* is lethal, at least on the medium used. In case this failure was an effect of genetic background, we also tried unsuccessfully to replace *wblE* in the independently derived

Table 2. *S. coelicolor* contains 11 chromosomal and three plasmid-located *wbl* genes, among which five are widely conserved among actinomycetes, whereas the rest are genus- or species-specific

<i>S. coelicolor</i> gene name	Length/pI of encoded protein		Equivalent genes in other actinobacteria*						Absent from†
	Length (aa)	pI	SCO	SAV	SCAB	SMD	SGR	<i>M. t. Rv</i>	
<i>wblA</i>	112	5.10	3579	4584	41391	05208	3340	3618c (<i>whiB4</i>)	Blo, Lxy, Tw
<i>whiB</i>	87	4.82	3034	5042	55081	04375	4503	3260c (<i>whiB2</i>)	
<i>wblC</i>	122	5.53	5190	3070	30701	017376	2235	3197A (<i>whiB7</i>)	Blo, Corynebacteria, Lxy, Pac, Tw
<i>whiD</i>	112	5.84	4767	4997	36241	06831	2760	3416 (<i>whiB3</i>)	Blo, Cdi, Lxy, Pac, Tw
<i>wblE</i>	85	7.79	5240	3016	30131	07459	2274	3219 (<i>whiB1</i>)	
<i>wblH</i>	81	5.09	6715	1693	13481	09520	1009	–	Most actinobacteria
<i>wblI</i>	125	10.17	5046	3216	33531	07193	2479	–	Most actinobacteria
<i>wblJ</i>	88	7.80	7106	–	–	–	–	–	Most actinobacteria
<i>wblK</i>	83	8.21	7306	–	–	–	6595	–	Most actinobacteria
<i>wblL</i>	106	5.00	6965a	–	12431	–	–	–	Most actinobacteria
<i>wblM</i>	213	9.31	6922	–	32261	08516	–	–	Most actinobacteria
<i>wblN</i>	102	8.56	SCP1.95	4490, 7514					Most actinobacteria
<i>wblO</i>	175	10.38	SCP1.115						Most actinobacteria
<i>wblP</i>	268	6.12	SCP1.161						Most actinobacteria

*Prefixes for gene identifiers: SCO, *S. coelicolor*; SAV, *S. avermitilis*; SCAB, *S. scabies*; SMD, *S. venezuelae*; SGR, *S. griseus*; *M. t. Rv*, *M. tuberculosis*.

†The genomes used in this analysis are those reviewed by Ventura *et al.* (2007), and the criterion for the presence of any gene in a genome is a reciprocal BLASTP hit with the relevant *S. coelicolor* gene. Abbreviations: Blo, *Bifidobacterium longum*; Cdi, *Corynebacterium diphtheriae*; Lxy, *Leifsonia xyli*; Pac, *Propionibacterium acnes*.

S. coelicolor A3(2) strains M600 and J1916. J1916 was the strain used in the successful disruption of *wblE* reported by Homerová *et al.* (2003). We did not study *wblE* further.

Confirming previous findings (Morris *et al.*, 2005), a *wblC* mutant was hypersensitive to diverse antibiotics, and had no obvious morphological change. None of the other eight single mutants was sensitive to any of a range of antibiotics, and just one of them, *wblA*, gave a phenotype clearly detectable on the complex medium R2YE, defined minimal medium (MM) or soy flour medium (MS) (see below). In case some of the supernumerary *wbl* genes were functionally redundant, we also constructed a series of double and triple mutant strains (*wblHK*, *IK*, *JK*, *KM*, *HIK*, *HJK*, *HKL*, *IJK*, *IKL*, *IKM*, *JKL*, *JKM*, *KLM*). None of these strains had any obvious growth defect or mutant morphological phenotype. The remainder of this paper therefore focuses on *wblA*.

On all media except MM containing glucose (on which development of the wild-type was relatively poor), the *wblA* mutant colonies displayed an unusual red pigmentation in their aerial mycelium (Fig. 1). The same 'red colony phenotype' was obtained with an unmarked in-frame *wblA* deletion (Fig. 1). The phenotype was complemented by the introduction of wild-type *wblA* DNA, either on cosmid H17-787 (integration at the ϕ C31 attachment site) or on cosmid H17-784 (single-crossover integration by homologous recombination) (Fig. 1). Since the reverse sides of *wblA* mutant patches

revealed much stronger pigmentation than the parental or complemented strains, it appears that pigmentation of lower parts of the mycelium was also increased by the mutation. Complementation was also obtained with the integrating plasmid pSET152::*wblA*, containing a *wblA*-specific fragment extending from 461 bp upstream of *wblA* to a few basepairs downstream (not shown). A different construct, containing *wblA* as a fragment extending only 81 bp upstream of the *wblA* coding sequence, failed to complement the mutant phenotype. This failure proved to be attributable to the absence of part of the major *wblA* promoter (see below).

Two pigmented antibiotics contribute to the red colony phenotype of *wblA* mutants

Production by *S. coelicolor* of two red-pigmented antibiotics, actinorhodin (Act, red in acidic conditions) and the prodiginine complex (Red), is usually localized to the parts of the substrate mycelium just below the aerial mycelium (Chater, 1998). To find out whether either antibiotic contributes to the red colour of the aerial mycelium, a *wblA*::*aac(3)IV* mutation on cosmid H17 was introduced by conjugation into strains unable to produce one or the other of them. Exconjugants were arrayed in patches, maintaining selection for Apra^R. In each case, about half of the patches were Kan^S and therefore likely to be double-crossover replacements. There was a complete correspondence between Kan^S and reddish aerial

Fig. 1. The red colony phenotype of *wblA* mutants, and its repair/complementation by cosmids carrying the wild-type *wblA* gene. Patches on MM + mannitol were incubated for 5 days. Left panel, upper side; right panel, underside. J3900 and J3907 were *wblA* deletion mutants. J3904, 3905 and 3906 were derived from J3900. J3904 contained the H17-787 cosmid (carrying *wblA*) inserted at the ϕ C31 attachment site, while the control strain J3905 carried the Supercos 1-787 vector with no insert. J3906 was typical of strains in which there had been a double-crossover replacement of $\Delta wblA::aac(3)IV$ by recombination with cosmid H17-784 [*pIJ784* cassette (*oriT*-*aac(3)IV*) targeted to the *bla* gene in the cosmid backbone]. The H17-784 cosmid was introduced into $\Delta wblA::aac(3)IV$ by conjugation. Both Apra^R Kan^R exconjugants (single-crossover integration, complementation, not shown) and Apra^S Kan^S (double-crossover replacement) segregants all had wild-type colony appearance.

mycelium, indicating that either of the two coloured antibiotics could cause the red colony phenotype; and in further confirmation, aerial mycelium of *wblA* red double mutants turned blue and excreted droplets of deep blue liquid on exposure to ammonia, a characteristic of Act (Fig. 2). The reverse sides of *wblA* red and *wblA* act double mutants both showed enhanced pigmentation (Fig. 2).

To confirm that Act and Red were the only direct causes of the red pigmentation of *wblA* mutant aerial mycelium, a similar experiment was carried out using as recipient an *act* red double mutant. Not only did Kan^S exconjugants fail to accumulate reddish pigments in any part of the colony, but their aerial mycelium lacked the grey pigment associated

with spore maturation, remaining white even on prolonged incubation (Fig. 2). Thus, *wblA* mutant aerial hyphae ectopically accumulated two pigmented antibiotics, but failed to accumulate detectable amounts of the polyketide spore pigment.

***wblA* mutation interferes drastically with aerial mycelium development**

Mutants lacking grey spore pigment are often sporulation-defective (Hopwood *et al.*, 1970; Chater, 1972). Consistent with this, harvesting of well-grown confluent plates of *wblA* mutants yielded viable counts 100- to 1000-fold lower than were obtained with complemented strains. We therefore used microscopic analysis to evaluate aerial mycelium development and sporulation of J3900.

In contrast to the abundant spore chains on the surface of wild-type colonies, phase-contrast microscopy of aerial hyphae of MM-grown J3900 colonies showed fields consisting almost entirely of long, fine, fairly straight unbranched hyphae, although some fields had a small number of apparently normal spore chains. SEM of the surface of J3900 colonies (grown on MS) likewise showed many long, unbranched and relatively thin aerial hyphae, with a diameter of about 0.5 µm (Fig. 3a), compared with 0.7 µm for the non-sporulated parts of aerial hyphae of the wild-type (Fig. 3d). Occasional fields contained spore chains of mostly normal length and dimensions (Fig. 3b). These spores appeared to be fully developed: they were covered by the basketwork-like array of paired rodlet structures typical of mature wild-type spores (Fig. 3c), and showed normal heat resistance (not shown). They did not represent reversion or suppression of the *wblA* mutation, since all the colonies growing after restreaking retained the typical *wblA* mutant phenotype. Thus, *WblA* is needed for sporulation only at a very early stage of aerial growth, and its role at this early stage can occasionally be bypassed.

SEM also revealed frequent thicker filaments ranging in diameter from 1 to 4 µm, and with relatively uneven surfaces, that were not seen in the parental control strain (Fig. 3b). These appeared to be bundles of individual hyphae embedded in, or covered by, extracellular material. Using TEM of J3900 colonies at the same stage, no spore chains were found, and the uppermost ~50 µm mainly consisted of thin hyphae (Fig. 4a), some of which were arranged in parallel bundles embedded in an electron-opaque matrix (Fig. 4a) that appeared fibrous at high magnification (Fig. 4b). In transverse section, these comprised from just a few up to ~100 individual hyphae. The hyphae in the bundles were often swollen, and were heterogeneous in appearance. The smaller bundles in the TEM images were mainly in the upper part of the aerial mycelium, and corresponded to the thicker filaments visible by SEM. In the mutant only, at the junction of the substrate mycelium and aerial mycelium there was a wide zone in which the spaces between hyphae were almost entirely filled with matrix material. Below this, the

Fig. 2. The red colony phenotype of a *wblA* mutant involves overproduction of both actinorhodin and undecylprodigiosin. The H17 $\Delta wblA::aac(3)/IV$ cosmid was mobilized into an actinorhodin-deficient mutant (M511), an undecylprodigiosin-deficient mutant (M510), and a double mutant lacking both antibiotics (M512). Apramycin-resistant colonies were picked to master-plates (MM plus mannitol) and incubated for 5 days before photographing them. The plates were also replicated to medium containing kanamycin (examples of sensitive colonies are marked with red or white arrows, and of resistant colonies with black arrows). With M510 and M511 there was a complete correspondence of the red colony phenotype with kanamycin sensitivity (loss of the cosmid, and replacement of the chromosomal copy of *wblA* by $\Delta wblA::aac(3)/IV$), and in both sets of plates, the mutant showed increased pigmentation on the underside (reversed plates shown on the right). Fuming of M510 *wblA* mutant patches with ammonia caused them to turn blue, as expected for actinorhodin. With M512, the Kan^S patches were devoid of all pigments, even the grey spore pigment.

Fig. 3. Scanning electron microscopy of the sporulation-deficient aerial mycelium of a *wblA* mutant (J3900). Images are of 6-day-old colonies grown on MS. (a) Most of the J3900 aerial mycelium is made up of non-sporulating unbranched thread-like hyphae. (b) Occasional spore chains in J3900, and an example of bundled aerial hyphae. (c) The surface of the spores in rare J3900 spore chains is covered with paired rodlets. (d) Typical field of the wild-type M145 aerial mycelium, showing stages in the development of spore chains.

substrate mycelium in the lowest parts of the colony resembled that of the wild-type, with occasional branches and cross-walls.

Transcription of *wblA* from multiple promoters

The developmental phenotype of *wblA* mutants raised the question of whether the *wblA* gene might itself be developmentally regulated. S1 mapping of RNA samples isolated from surface cultures of M145 at time points covering different stages of differentiation showed that the most abundant *wblA* mRNA 5'-end was approximately 65 bp upstream of the start codon, with two less abundant ones further upstream (at approximately -331 and -391) (Fig. 5). Transcription from the strong downstream promoter P3 was about equal at all the time points up to 72 h, after which it diminished. The two less abundant mRNA species (from P1 and P2) were also most readily seen at the earliest time points, but diminished earlier than transcripts from P3. In a previous study, the same RNA samples were shown to contain very low levels of mRNA for the sporulation-specific *whiA*, *whiB*, *whiH* and *whiI* genes at time points before 48 h, but higher levels from 48 h onwards, while mRNA for the constitutive *hrdB* gene was present at roughly similar levels at all time points (Jakimowicz *et al.*, 2006). It therefore appears that *wblA*

mRNA is abundant in mycelial cell-types other than aerial hyphae undergoing sporulation, consistent with the evidence above showing that although WblA is important for the initiation of sporulation, it does not have a significant role in the sporulation process per se.

The sequence GTTG-(N₁₅)-CCGTA found upstream of the position corresponding to the major *wblA* mRNA 5'-end (P3, Fig. 5) is similar to the consensus sequence GTTG-(N₁₅₋₁₇)-GGGTA for mycobacterial σ^F -dependent promoters, which include the promoter of the *wblA* orthologue *whiB4* in *M. smegmatis* (Hümpel *et al.*, 2010). This suggests that *wblA*P3 is recognized by one or more of the nine σ^F -like sigma factors of *S. coelicolor*, many of which are involved in stress responses and development (e.g. Dalton *et al.*, 2007).

Expression of a large number of genes is affected by *wblA* mutation

The wide-ranging phenotypic effects of *wblA* mutation suggested significant changes in global gene expression in the mutants. We therefore compared the transcriptomes of a *wblA* mutant (J3900) and the wild-type parent (M145) during growth and development, using triplicate cultures (biological replicates) grown on cellophane overlays on

Fig. 4. Transmission electron micrographs showing aerial hyphae of a *wblA* mutant (J3900). Images are thin sections of 6-day-old colonies fixed in glutaraldehyde/osmium tetroxide and post-stained with uranyl acetate/lead. (a) Aerial hyphae of the mutant were often bundled together in an extracellular matrix. (b) At high magnification the matrix appeared fibrous. (c) Wild-type (M145) aerial mycelium showed frequent dark spore chains, and the aerial hyphae were never bundled in a matrix.

MM + mannitol for various times of incubation up to 84 h. In M145 during this time, a period of vegetative growth (represented by 24 h and 36 h RNA samples) was followed by slower biomass accumulation accompanied by aerial growth (48 h and 60 h RNA samples), and then by growth cessation and sporulation (from 60 h) (Fig. 6a). The early growth of the *wblA* mutant was similar but, unlike M145, the mutant continued to grow rapidly between 48 and 60 h, and achieved higher biomass. The strong pigmentation typical of *wblA* mutants began at about 40 h, while M145 accumulated only very low levels of pigment.

In the mutant, using the stringent criteria described in Methods, 183 genes showed increased expression, 103 decreased expression, and five increased expression at one or more time points and decreased expression at others (Table 3 and Supplementary Fig. S1). More than 75 % of the 291 genes showed the maximum mutant/wild-type difference at 48 h or 60 h, corresponding to the time when the phenotypic differences between the mutant and its parent became obvious (Fig. 6). However, some maxima were earlier, suggesting that *WblA* also influences gene expression before overt differentiation begins, consistent with its early expression (Fig. 5). Regulatory and signal transduction genes made up 25 of overexpressed genes and 24 of underexpressed genes (Table 3), perhaps accounting for the major transcriptional changes resulting from *WblA* elimination.

In line with the delay in shutting down vegetative growth, numerous primary metabolism genes were overexpressed

in the mutant (Table 3). Thus, 15 genes associated with the biosynthesis of amino acids and cofactors were overexpressed in J3900, while no such genes were down-regulated; five genes for central carbon metabolism were overexpressed, and none downregulated; seven fatty acid biosynthetic genes were overexpressed compared with one underexpressed; and several genes encoding likely components of uptake systems for amino acids or ammonia were overexpressed. The red colony phenotype correlated with increased expression of genes for actinorhodin production, including the pathway-linked regulatory gene *actII orf4*. Effects of *wblA* mutation were also noted on some other genes for secondary metabolism.

As might be expected, some genes concerned with morphological development were underexpressed in J3900. They included several genes for chaplins, the amphipathic proteins that provide aerial hyphae with a hydrophobic outer layer (Willey *et al.*, 2006), as well as one of several genes for sortases, which are thought to anchor long chaplins to the cell wall (Elliot *et al.*, 2003; Claessen *et al.*, 2003). Other developmental genes showing *wblA* dependence included *nepA*, a marker for the earliest known hyphal cell-type in the formation of reproductive aerial mycelium (Dalton *et al.*, 2007); *smeA*, which encodes a small membrane protein that targets an FtsK-like protein to sporulation septa (Ausmees *et al.*, 2007); and *whiB*, an early sporulation gene that is the prototypical *wbl* gene (Davis & Chater, 1992), raising the possibility of *Wbl*-to-

Fig. 5. S1 nuclease protection analysis of *wblA* transcription. RNA from surface-grown cultures (MS agar medium), harvested at the time points indicated in hours above the tracks, was used to protect the radiolabelled 5'-end of a DNA probe against digestion with S1 nuclease. After electrophoresis on a polyacrylamide gel, short (left) and longer (right) autoradiographic exposures were made. M, molecular size standards (sizes in nt given next to the bands). The approximate lengths of the three main protected species are indicated in nucleotides. To the right of the autoradiographs, the sequence of the *wblA* region is shown, annotated as follows: boxes, coding sequences (putative start codon shaded); asterisk arrow, radiolabelled primer used in PCR to amplify probe for S1 mapping (other primer not shown); bent arrows, approximate positions of mRNA 5'-ends; P1, P2, P3, putative promoter designations; underlined sequences, close matches to the consensus sequence for promoters recognized by σ^F in *M. tuberculosis* (see Results). The onset of aerial mycelium formation and sporulation are indicated by arrows marked 'am' and 's', respectively.

Wbl regulation. Reduced WhiB abundance might account for some aspects of the sporulation-defective phenotype.

Less expectedly, some genes with significant roles in aerial growth were overexpressed in J3900. These included *ramS*, the structural gene for the precursor of SapB, a secreted surfactant peptide that, like chaplins, aids aerial growth; SCO0762, encoding a secreted protease inhibitor (Sti) that holds in check an extracellular protease cascade implicated in development (Kim *et al.*, 2008); and *ssgR*, encoding a nutritionally sensitive regulator of the adjacent gene, *ssgA*, which is involved in the placement of septa in both *S. coelicolor* and *Streptomyces griseus* (van Wezel *et al.*, 2000; Traag *et al.*, 2004; Jiang & Kendrick, 2000; Willemse *et al.*, 2011).

DISCUSSION

Comparative genomics of *wbl* genes in relation to function

Of the 11 chromosomal *wbl* genes of *S. coelicolor*, six (*wblH*, *-I*, *-J*, *-K*, *-L*, *-M*) could be mutated without obvious phenotypic effects, even in various double and triple mutant combinations. Orthologues of these six genes are generally absent from other actinomycetes, and only *wblH* and *wblI* are present in most *Streptomyces* genomes sequenced. Five of them (the exception being *wblI*) are located in the right-hand end of the linear chromosome, which consists very largely of species-specific genes that have probably mostly been acquired by lateral gene transfer

Fig. 6. The effects of *wblA* mutation are generally strongest at the time of aerial mycelium formation. (a) Levels of protein isolated from cultures of M145 (black line) and the *wblA* mutant (J3900, grey line) at the times indicated. The values are the means of duplicate experiments with cultures grown under the same conditions as those used to prepare RNA for the microarray experiment. The times of initiation of aerial mycelium formation and sporulation are indicated. (b) Histogram showing how many of the 291 genes significantly influenced by *wblA* deletion showed the highest difference between the wild-type and mutant abundance levels at the time points indicated, as determined by microarray analysis of RNA. Black, genes with increased expression in the mutant; grey, genes with decreased expression in the mutant.

(e.g. Choulet *et al.*, 2006; Ventura *et al.*, 2007) and may have adaptive value mainly in ecological or physiological circumstances that are encountered only intermittently.

In contrast, the five most widely conserved *wbl* genes all lie in the central region of the genome, along with most of the genes conserved among streptomycetes (including *wblI*); and these *wbl* genes all have some synteny with nearby genes, in some cases extending to other genera of actinobacteria (our unpublished observations). The roles of *whiB* and *whiD* in sporulation and *wblC* in resistance to antibiotics and other toxic metabolites were established previously (Davis & Chater 1992; Molle *et al.*, 2000; Morris *et al.*, 2005). In our hands a fourth, *wblE*, could not be eliminated, and therefore appears to be essential for the strains and conditions employed here, although it was reported by Homarová *et al.* (2003) to be possible to eliminate it without phenotypic effects. We

speculate that this different result might be attributable to the powerful selection that they used (which could have allowed the emergence of a suppressor mutation), or to the fact that their mutant retained part of the *wblE* gene.

Mutation of the fifth conserved gene, *wblA*, reported here for the first time, resulted in a completely novel phenotype: overproduction of pigmented antibiotics, and their ectopic presence in the aerial mycelium; a general failure of aerial hyphae to exhibit any aspects of sporulation, except that a few spore chains mostly of wild-type appearance were formed; and the embedding of bundles of aerial hyphae in an extracellular matrix. Detailed study also revealed a delay in shutting off rapid growth as nutrients approach exhaustion. Since mutations in the archetypal *wbl* gene, *whiB*, also prevent controlled growth cessation (in that case of aerial hyphae: Flärdh *et al.*, 1999), it is possible that other, phenotypically cryptic, *wbl* mutations might subtly affect some aspect of growth control.

Involvement of WblA in the transition from primary to secondary metabolism

The prolongation of rapid growth caused by *wblA* mutation was accompanied by overexpression of several genes associated with primary metabolism. These included *argH*, *B* and *J*; *pheA*; genes for branched-chain amino acid transport; *amtB* and the adjacent gene *glnK*, respectively encoding the ammonia transporter and the PII signal transduction protein, which transmits information about nitrogen availability from AmtB; and several genes for glutamine/glutamate-metabolizing enzymes (an area of metabolism that is the first affected when liquid glutamate-grown cultures begin to sense nutrient stress: Nieselt *et al.*, 2010). As noted by Hodgson (2000), some of the biosynthetic genes for arginine, branched-chain amino acids and aromatic amino acids are unusual among genes for amino acid biosynthesis in *S. coelicolor* in being subject to end-product feedback repression; so WblA may play some part in this regulation. It is possible that the effects of *wblA* mutation on colony differentiation and secondary metabolism may be secondary consequences of the changes in primary metabolism.

Recently, Kim *et al.* (2011) found that the red colony phenotype of one of our *wblA* mutants is mirrored in substantially increased actinorhodin production in liquid fermentations. Actinorhodin overproduction by J3900 is probably at least partially attributable to overexpression of the pathway-associated regulatory gene *actII orf4*. This may in turn be a consequence of overexpression of *afsS*, encoding a small, somewhat sigma-factor-like, protein that mediates nutritional stress-responsive activation of antibiotic production (Lian *et al.*, 2008). Additionally, it was previously reported that deletion of the *catB* (catalase) gene, SCO0666, which was underexpressed in the *wblA* mutant, results in actinorhodin hyperproduction (Cho *et al.*, 2000, 2001).

Table 3. Genes influenced by deletion of *wblA*, based on microarray analysis

Abbreviations: aa, amino acid; Ac, acetyltransferase; Am, aminotransferase; HK, histidine protein kinase; Me, methyltransferase; PBP, penicillin-binding protein; RR, response regulator; STK, serine/threonine protein kinase.

Functional class	Up in <i>wblA</i> mutant (SCO numbers, annotated as appropriate)	Down in <i>wblA</i> mutant (SCO numbers, annotated as appropriate)
Regulation and signal transduction (excluding developmental functions)	0143, 0193, 0551 (HK), 0669, 1160 (<i>cvnA3</i> , nitrate-sensing HK), 2805 (<i>orf1590</i> -like), 3900, 4198, 4214 (anti-sigma?), 4507 (STK), 5036, 5217 (anti-sigma RsrA), 5584 (PII), 5616, 5804 (ribonucleotide reductase), 6153, 7067, 7446 (RsbW-like), 7640, 7759 (SpoIIE-like)	0588 (<i>cvnA11</i> , HK), 0822, 0840, 0898 (AfsR-like), 1241 (anti-sigma?), 1628 (<i>cvnC9</i>), 1658 (<i>gylR</i>), 2333 (<i>bldB</i> -like), 2398, 2846, 2958 (RR), 3583, 3857, 4276 (RR, SenR), 4313, 4442 (<i>bldB</i> -like), 4488 (STK), 5287, 5338, 5678, 6895, 7291, 7438
Macromolecular synthesis and processing	2615 (<i>vals</i>), 3429 (<i>rpmB</i>), 4649 (ribosomal protein L1), 4735 (ribosomal protein S9)	1508 (<i>hisS</i>), 5184 (helicase?)
Cell wall biosynthesis	2689 (glycosyltransferase), 3122 (<i>gluM</i>), 3901 (PBP)	5467 (pentapeptide carboxypeptidase)
Energy metabolism	2488 (<i>nirC</i>), 4858 (succinate dehydrogenase), 4948 (<i>narH3</i>), 4950 (<i>narI3</i>), 5106 (succinate dehydrogenase)	1934 (cytochrome oxygenase assembly factor), 2559 (coproporphyrinogen III oxidase)
Redox	0999 (<i>sodF2</i>)	0666 (<i>catB</i>)
Transport and membrane proteins, and lipoproteins	0079, 0416, 0708 (branched-chain aa transport), 1632 (<i>tatC</i>), 1820, 1900, 1903, 2008 (branched-chain aa transport), 2641, 2809, 2978 (binding protein?), 3418 (ABC transporter), 3663, 3720, 3999, 4011, 4509 (ESAT-6), 4534, 4867, 4885, 4934, 4963, 5022, 5035, 5259 (<i>atrB</i>), 5428, 5458, 5477, 5478, 5479, 5500, 5579, 5583 (<i>amtB</i>), 5866, 6141, 6259, 6822	0464, 0849, 0853, 1195, 1411, 1418, 1457, 1786, 2498, 2629, 2842, 3019, 3515, 3620, 3708, 3787, 3789, 4237, 4374, 4693, 4854, 5110, 5266, 6054, 7007, 7434
Secreted proteins	2239, 2663, 2741, 2762, 2837, 2892, 6557 (neuraminidase?), 7233, 7657, 7661	0743, 0931, 1444, 2841 (sortase), 2926
Amino acid, purine, pyrimidine, cofactor biosynthesis	1245 (<i>bioA</i>), 1570 (<i>argH</i>), 1578 (<i>argB</i>), 1579 (<i>argJ</i>), 1849 (<i>cobN</i>), 2026 (<i>gltB</i>), 2241 (glutamine synthase), 3381 (<i>nadC</i>), 3629 (<i>purA</i>), 3962 (<i>pheA</i>), 4079 (<i>purL</i>), 4563 (<i>nuoB</i>), 5140 (lysine decarboxylase), 5374 (<i>atpC</i>), 5845 (thymidine kinase)	
Central carbon metabolism	4388 (<i>citA</i>), 5047 (<i>glpX</i>), 5059 (<i>ppgK</i>), 5423 (<i>pyk2</i>), 5426 (<i>pfkA</i>)	
Fatty acid metabolism	1814 (<i>fabI</i>), 1831 (<i>fabG</i> -like), 2388 (<i>fabH</i>), 3246 (<i>fabH4</i>), 4921 (<i>accA2</i>), 6282 (<i>fabG</i> -like), 6791 (acyl-CoA dehydrogenase)	3248 (<i>fabF3</i>)
Catabolic pathways	2786 (acetylhexosaminidase <i>hexA</i>), 2999 (glutamate dehydrogenase), 4089 (<i>vdh</i>), 4257 (carbohydrate hydrolase?), 6213 (atrazine hydrolase?) 6284 (decarboxylase), 7471 (<i>paaA</i>)	1592 (ADP ribose pyrophosphatase), 1773 (alanine dehydrogenase), 4859 (purine catabolism), 6800 (threonine catabolism)
Proteases	4670, 5179, 5180, 5446, 6995	2266 (<i>map3</i>)
Actinorhodin biosynthesis	5071, 5073, 5076, 5077, 5080, 5081, 5085, 5088, 5089	
Miscellaneous secondary metabolism	2785 (siderophore biosynthesis?) 3230 (CDA peptide synthase)	1866 (<i>ectC</i>), 5176 (epimerase/dehydratase?), 7458 (transglycosylase?)
Transferases	0222 (Ac), 0777 (GST), 4111 (Me)	0603 (Am), 2170 (Me), 5972 (Me), 7062 (Me)
Oxidoreductase	0252, 0318, 3092, 6042 (chlorite dismutase), 6712 (Cu oxidase?)	2940
Developmental/structural (not regulatory)	0762 (Sti), 4253 (tail sheath), 5038 (flotillin), 6682 (<i>ramS</i>), 7257 (<i>chpB</i>)	0650 (<i>gvpA2</i>), 0654 (<i>gvpZ2</i>), 1415 (<i>smeA</i>), 1800 (<i>chpE</i>), 2716 (<i>chpA</i>), 2717 (<i>chpD</i>), 4002 (<i>nepA</i>)
Developmental regulatory, <i>wbl</i>	3925 (<i>ssgR</i>), 4425 (<i>afsS</i>), 7106 (<i>wblJ</i>)	3034 (<i>whiB</i>)
Transposons/plasmids/phages	0076, 5349, 6337	5610, 6393

Table 3. cont.

Functional class	Up in <i>wblA</i> mutant (SCO numbers, annotated as appropriate)	Down in <i>wblA</i> mutant (SCO numbers, annotated as appropriate)
Hypotheticals	0149, 0178, 0225, 0227, 1041, 1340, 1795, 1841, 2134, 2167, 2211, 2316, 3215, 3309, 3726, 4003, 4028, 4060, 4199, 4287, 4335, 4427, 4821, 4873, 4980, 4992, 5000, 5020, 5037, 5191, 5312, 5389, 5726, 6238, 6437, 6593, 6923, 7608	0833, 0834, 0838, 0854, 1783, 1987, 2364, 3324, 3379, 4144, 4676, 6072, 6142, 6177, 6200, 6887, 7065, 7247
Unclassified	2562 (<i>lepA</i>), 4295 (cold shock <i>scoF4</i>)	

Surprisingly, no overexpression of genes for undecylprodigiosin biosynthesis was detected, even though this red pigment was shown to be able to cause a red colony phenotype in a *wblA act* double mutant. Regulatory cross-talk between disparate antibiotic pathways may account for this paradox (Huang *et al.*, 2005; Xu *et al.*, 2010).

WblA may facilitate the subdivision of aerial hyphal initials into developmentally distinct apical and subapical compartments

WblA has an important role very early in aerial hyphal development. The thread-like non-sporulating aerial hyphae of the *wblA* mutant are thinner than normal aerial hyphae or the straight, non-sporulating aerial hyphae of *whiG* mutants (Fig. 3a; Chater, 1972), and unlike the latter do not contain frequent cross-walls or exhibit short side branches (Plaskitt & Chater, 1995; Flärdh *et al.*, 1999). Rather, they seem more similar to those of a mutant defective in the cell division activator gene *ssgA*. Strikingly, *ssgA* mutants also give low numbers of spores on mannitol-containing media (van Wezel *et al.*, 2000; Jiang & Kendrick, 2000).

There is usually a short narrower segment at the base of wild-type aerial hyphae (e.g. Chater, 1972), so the *wblA* mutant aerial hyphae may fail to progress from this state. We suggest that WblA may help to bring about the early division, by a specialized basal septum, of the emerging aerial hypha into two compartments: a thin subapical stem compartment fated eventually to die (Dalton *et al.*, 2007), and the wider apical compartment fated to develop into a chain of spores. On this model, formation of the basal septum would be a checkpoint for further developmental progression, so the growing aerial hyphae of a *wblA* mutant might continue to manifest the physiology of the aerial hyphal initial compartment. A specialized basal septum was demonstrated in *S. griseus* by Kwak *et al.* (2001), and a conserved gene for a predicted Pbp1B-like peptidoglycan transglycosylase/transpeptidase is found next to *wblA* orthologues in streptomycetes (e.g. SCO3580) and several other actinobacteria. If a different kind of septum, such as a vegetative septum, were to form ectopically in a nascent *wblA* mutant aerial hypha, this checkpoint could be passed, and sporulation of that hypha would result. This may explain the occasional spore chains

in J3900 colonies. Failure to form a proper subapical stem compartment was indicated by the underexpression in the *wblA* mutant of the subapical stem compartment marker gene *nepA* (Dalton *et al.*, 2007) (note that that *nepA* may also be expressed in wild-type apical compartments: de Jong *et al.*, 2009a).

Bundling of aerial hyphae and its occurrence in other genotypes

Bundling of aerial hyphae was also reported in a *nepA* mutant (de Jong *et al.*, 2009a), so the bundling of *wblA* mutant aerial hyphae might be a consequence of the low *nepA* expression in the mutant. Similar bundled aerial hyphae, giving white aerial mycelium and infrequent spores, also occurred when multiple copies of a gene cluster termed *sas* were introduced into *S. griseus* (Takano *et al.*, 2008). However, the possible orthologues of the *sas* cluster in *S. coelicolor* (SCO0682–5) did not show changed expression in the microarray analysis of the *wblA* mutant.

The matrix of bundles resembles cellulose in its fibrillar appearance. Cellulose-like material is known to be present both at aerial hyphal tips, possibly facilitating the maturation of the hydrophobic sheath material that surrounds aerial hyphae, and in association with fimbria-like structures implicated in the chaplin-mediated adhesion of vegetative hyphae to their substratum (Del Sol *et al.*, 2007; Xu *et al.*, 2008; de Jong *et al.*, 2009b; Chater *et al.*, 2010). We suggest that the production of glucan may be a normal function of aerial hyphal initials, helping their growth away from the substrate, while a glucan turnover function may be active only after the compartmentalization of the aerial hyphae. Thus, the uncompartimentalized aerial hyphae of the *wblA* mutant would continuously produce glucan (Fig. 7). One of the most strongly overexpressed genes in J3900 was SCO0762, whose product inhibits an extracellular proteolytic cascade that plays a role in development, possibly by activating proenzymes (see Chater *et al.*, 2010, for a review). Overproduction of SCO0762 protein would be expected to retard some aspects of development, because it would inhibit the autolytic degradation of non-sporulating parts of the mycelium, which provides nutrients for the production of spores. Perhaps, *inter alia*, the proteolytic cascade activates an enzyme for degradation of the extracellular matrix (Fig. 7).

Fig. 7. Schematic diagram of the effects of *wblA* mutation on early stages in aerial hyphal development.

WblA affects the expression of genes for aerial hyphal structure-determining proteins

The microarray data suggested that the abnormally extended mutant initial aerial hyphal compartment proposed for *wblA* mutants expresses genes for some of the surface components of aerial hyphae: *ramS* (encoding pro-SapB) and *chpB* (encoding one of the large chaplins). This may implicate WblA in the non-production of SapB by the wild-type on minimal medium, conditions under which most chaplins are strongly produced (Capstick *et al.*, 2007). Other genes potentially involved in determining structures were overexpressed in J3900: SCO4253, encoding a protein resembling phage tail sheath proteins; and SCO5038, one of several similar genes present in the genome that encode proteins similar to eukaryotic flotillins (Table 3). Eukaryotic flotillins are associated with lipid rafts, where they serve as a nucleus for the assembly of protein complexes; and similar proteins occur in many bacteria (Langhorst *et al.*, 2005), where they are proving to play a similar role, in some cases with developmental implications (Donovan & Bramkamp, 2009; López & Kolter, 2010). Although Fig. 7 interprets the apparent

overexpression of these various organizing or structure-determining genes to mean that they are normally repressed, directly or indirectly, by WblA in the aerial hyphal initial branches, it is also possible that the increased abundance of their transcripts reflects the increased contribution of this cell-type to the overall biomass of the colonies.

Some other chaplin genes (*chpA*, *chpD*, *chpE*) were under-expressed in J3900, as were a few other genes with the potential to specify components of self-assembling structures, such as several in a gene cluster for gas vesicle protein synthesis (SCO0649–0658, of which SCO0650 and SCO0654 met the criteria for WblA-dependence) (van Keulen *et al.*, 2005). The two rodlin genes SCO2718–9 (Claessen *et al.*, 2002, 2004) were also underexpressed in the mutant, but did not meet the criteria adopted for inclusion. An earlier microarray study (Elliot *et al.*, 2003) found that most chaplin and rodlin genes and *nepA* were also among the 17 most clearly downregulated genes in a mutant defective in *bldN*, which encodes a developmentally significant sigma factor (Bibb *et al.*, 2000), while changes in the expression of *chpB*, the only 'WblA-repressible' chaplin

gene, were not found in a *bldN* mutant (Elliot *et al.*, 2003). ChpB may be developmentally distinct from the other chaplins.

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REFERENCES

- Alam, M. S., Garg, S. K. & Agrawal, P. (2007). Molecular function of WhiB4/Rv3681c of *Mycobacterium tuberculosis* H37Rv: a [4Fe-4S] cluster co-ordinating protein disulphide reductase. *Mol Microbiol* **63**, 1414–1431.
- Alam, M. S., Garg, S. K. & Agrawal, P. (2009). Studies on structural and functional divergence among seven WhiB proteins of *Mycobacterium tuberculosis* H37Rv. *FEBS J* **276**, 76–93.
- Ausmees, N., Wahlstedt, H., Bagchi, S., Elliot, M. A., Buttner, M. J. & Flärdh, K. (2007). SmeA, a small membrane protein with multiple functions in *Streptomyces* sporulation including targeting of a SpoIIIE/FtsK-like protein to cell division septa. *Mol Microbiol* **65**, 1458–1473.
- Bentley, S. D., Chater, K. F., Cerdeño-Tárraga, A. M., Challis, G. L., Thomson, N. R., James, K. D., Harris, D. E., Quail, M. A., Kieser, H. & other authors (2002). Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**, 141–147.
- Bentley, S. D., Brown, S., Murphy, L. D., Harris, D. E., Quail, M. A., Parkhill, J., Barrell, B. G., McCormick, J. R., Santamaria, R. I. & other authors (2004). SCP1, a 356,023 bp linear plasmid adapted to the ecology and developmental biology of its host, *Streptomyces coelicolor* A3(2). *Mol Microbiol* **51**, 1615–1628.
- Bibb, M. J., Molle, V. & Buttner, M. J. (2000). σ^{bldN} , an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2). *J Bacteriol* **182**, 4606–4616.
- Bierman, M., Logan, R., O'Brien, K., Seno, E. T., Rao, R. N. & Schoner, B. E. (1992). Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* **116**, 43–49.
- Bucca, G., Brassington, A. M., Hotchkiss, G., Mersinias, V. & Smith, C. P. (2003). Negative feedback regulation of *dnaK*, *clpB* and *lon* expression by the DnaK chaperone machine in *Streptomyces coelicolor*, identified by transcriptome and in vivo DnaK-depletion analysis. *Mol Microbiol* **50**, 153–166.
- Capstick, D. S., Willey, J. M., Buttner, M. J. & Elliot, M. A. (2007). SapB and the chaplins: connections between morphogenetic proteins in *Streptomyces coelicolor*. *Mol Microbiol* **64**, 602–613.
- Chater, K. F. (1972). A morphological and genetic mapping study of white colony mutants of *Streptomyces coelicolor*. *J Gen Microbiol* **72**, 9–28.
- Chater, K. F. (1998). Taking a genetic scalpel to the *Streptomyces* colony. *Microbiology* **144**, 1465–1478.
- Chater, K. F. & Chandra, G. (2006). The evolution of development in *Streptomyces* analysed by genome comparisons. *FEMS Microbiol Rev* **30**, 651–672.
- Chater, K. F., Biró, S., Lee, K. J., Palmer, T. & Schrepf, H. (2010). The complex extracellular biology of *Streptomyces*. *FEMS Microbiol Rev* **34**, 171–198.
- Cho, Y. H., Lee, E. J. & Roe, J. H. (2000). A developmentally regulated catalase required for proper differentiation and osmoprotection of *Streptomyces coelicolor*. *Mol Microbiol* **35**, 150–160.
- Cho, Y. H., Lee, E. J., Ahn, B. E. & Roe, J. H. (2001). SigB, an RNA polymerase sigma factor required for osmoprotection and proper differentiation of *Streptomyces coelicolor*. *Mol Microbiol* **42**, 205–214.
- Choulet, F., Aigle, B., Gallois, A., Mangelot, S., Gerbaud, C., Truong, C., Francou, F.-X., Fourier, C., Guérineau, M. & other authors (2006). Evolution of the terminal regions of the *Streptomyces* linear chromosome. *Mol Biol Evol* **23**, 2361–2369.
- Claessen, D., Wösten, H. A. B., van Keulen, G., Faber, O. G., Alves, A. M. C. R., Meijer, W. G. & Dijkhuizen, L. (2002). Two novel homologous proteins of *Streptomyces coelicolor* and *Streptomyces lividans* are involved in the formation of the rodlet layer and mediate attachment to a hydrophobic surface. *Mol Microbiol* **44**, 1483–1492.
- Claessen, D., Rink, R., de Jong, W., Siebring, J., de Vreugd, P., Boersma, F. G. H., Dijkhuizen, L. & Wösten, H. A. B. (2003). A novel class of secreted hydrophobic proteins is involved in aerial hyphae formation in *Streptomyces coelicolor* by forming amyloid-like fibrils. *Genes Dev* **17**, 1714–1726.
- Claessen, D., Stokroos, I., Deelstra, H. J., Penninga, N. A., Bormann, C., Salas, J. A., Dijkhuizen, L. & Wösten, H. A. B. (2004). The formation of the rodlet layer of streptomycetes is the result of the interplay between rodlets and chaplins. *Mol Microbiol* **53**, 433–443.
- Crack, J. C., den Hengst, C. D., Jakimowicz, P., Subramanian, S., Johnson, M. K., Buttner, M. J., Thomson, A. J. & Le Brun, N. E. (2009). Characterization of [4Fe-4S]-containing and cluster-free forms of *Streptomyces* WhiD. *Biochemistry* **48**, 12252–12264.
- Crack, J. C., Smith, L. J., Stapleton, M. R., Peck, J., Watmough, N. J., Buttner, M. J., Buxton, R. S., Green, J., Oganessian, V. S. & other authors (2011). Mechanistic insight into the nitrosylation of the [4Fe-4S] cluster of WhiB-like proteins. *J Am Chem Soc* **133**, 1112–1121.
- Dalton, K. A., Thibessard, A., Hunter, J. I. & Kelemen, G. H. (2007). A novel compartment, the 'subapical stem' of the aerial hyphae, is the location of a *sigN*-dependent, developmentally distinct transcription in *Streptomyces coelicolor*. *Mol Microbiol* **64**, 719–737.
- Datsenko, K. A. & Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* **97**, 6640–6645.
- Davis, N. K. & Chater, K. F. (1992). The *Streptomyces coelicolor* whiB gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. *Mol Gen Genet* **232**, 351–358.
- de Jong, W., Manteca, A., Sanchez, J., Bucca, G., Smith, C. P., Dijkhuizen, L., Claessen, D. & Wösten, H. A. (2009a). NepA is a structural cell wall protein involved in maintenance of spore dormancy in *Streptomyces coelicolor*. *Mol Microbiol* **71**, 1591–1603.
- de Jong, W., Wösten, H. A. B., Dijkhuizen, L. & Claessen, D. (2009b). Attachment of *Streptomyces coelicolor* is mediated by amyloid fimbriae that are anchored to the cell surface via cellulose. *Mol Microbiol* **73**, 1128–1140.
- Del Sol, R., Armstrong, I., Wright, C. & Dyson, P. (2007). Characterization of changes to the cell surface during the life cycle of *Streptomyces coelicolor*: atomic force microscopy of living cells. *J Bacteriol* **189**, 2219–2225.
- den Hengst, C. D. & Buttner, M. J. (2008). Redox control in actinobacteria. *Biochim Biophys Acta* **1780**, 1201–1216.
- Di Berardo, C., Capstick, D. S., Bibb, M. J., Findlay, K. C., Buttner, M. J. & Elliot, M. A. (2008). Function and redundancy of the chaplin cell surface proteins in aerial hypha formation, rodlet assembly, and viability in *Streptomyces coelicolor*. *J Bacteriol* **190**, 5879–5889.

- Donovan, C. & Bramkamp, M. (2009). Characterization and subcellular localization of a bacterial flotillin homologue. *Microbiology* **155**, 1786–1799.
- Elliot, M. A., Karoonuthaisiri, N., Huang, J. Q., Bibb, M. J., Cohen, S. N., Kao, C. M. & Buttner, M. J. (2003). The chaplins: a family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. *Genes Dev* **17**, 1727–1740.
- Flårdh, K., Findlay, K. C. & Chater, K. F. (1999). Association of early sporulation genes with suggested developmental decision points in *Streptomyces coelicolor* A3(2). *Microbiology* **145**, 2229–2243.
- Florian, B. & Bibb, M. J. (1996). *afsR* is a pleiotropic but conditionally required regulatory gene for antibiotic production in *Streptomyces coelicolor* A3(2). *Mol Microbiol* **21**, 385–396.
- Gao, B., Paramanathan, R. & Gupta, R. S. (2006). Signature proteins that are distinctive characteristics of Actinobacteria and their subgroups. *Antonie van Leeuwenhoek* **90**, 69–91.
- Garg, S. K., Suhail Alam, M., Soni, V., Radha Kishan, K. V. & Agrawal, P. (2007). Characterization of *Mycobacterium tuberculosis* WhiB1/Rv3219 as a protein disulfide reductase. *Protein Expr Purif* **52**, 422–432.
- Geiman, D. E., Raghunand, T. R., Agarwal, N. & Bishai, W. R. (2006). Differential gene expression in response to exposure to antimycobacterial agents and other stress conditions among seven *Mycobacterium tuberculosis* whiB-like genes. *Antimicrob Agents Chemother* **50**, 2836–2841.
- Gomez, J. E. & Bishai, W. R. (2000). *whmD* is an essential mycobacterial gene required for proper septation and cell division. *Proc Natl Acad Sci U S A* **97**, 8554–8559.
- Guo, M., Feng, H., Zhang, J., Wang, W., Wang, Y., Li, Y., Gao, C., Chen, H., Feng, Y. & He, Z. G. (2009). Dissecting transcription regulatory pathways through a new bacterial one-hybrid reporter system. *Genome Res* **19**, 1301–1308.
- Gusarov, I., Shatalin, K., Starodubtseva, M. & Nudler, E. (2009). Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. *Science* **325**, 1380–1384.
- Gust, B., Challis, G. L., Fowler, K., Kieser, T. & Chater, K. F. (2003). PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci U S A* **100**, 1541–1546.
- Gust, B., Chandra, G., Jakimowicz, D., Yuqing, T., Bruton, C. J. & Chater, K. F. (2004). Lambda red-mediated genetic manipulation of antibiotic-producing *Streptomyces*. *Adv Appl Microbiol* **54**, 107–128.
- Gust, B., O'Rourke, S., Bird, N., Kieser, T. & Chater, K. (2006). *Recombineering in Streptomyces coelicolor*. FEMS Online Protocols.
- Hodgson, D. A. (2000). Primary metabolism and its control in streptomycetes: a most unusual group of bacteria. *Adv Microb Physiol* **42**, 47–238.
- Homerová, D., Sevcíková, J. & Kormanec, J. (2003). Characterization of the *Streptomyces coelicolor* A3(2) *wblE* gene, encoding a homologue of the sporulation transcription factor. *Folia Microbiol (Praha)* **48**, 489–495.
- Hopwood, D. A., Wildermuth, H. & Palmer, H. M. (1970). Mutants of *Streptomyces coelicolor* defective in sporulation. *J Gen Microbiol* **61**, 397–408.
- Huang, J., Shi, J., Molle, V., Sohlberg, B., Weaver, D., Bibb, M. J., Karoonuthaisiri, N., Lih, C. J., Kao, C. M. & other authors (2005). Cross-regulation among disparate antibiotic biosynthetic pathways of *Streptomyces coelicolor*. *Mol Microbiol* **58**, 1276–1287.
- Hümpel, A., Gebhard, S., Cook, G. M. & Berney, M. (2010). The SigF regulon in *Mycobacterium smegmatis* reveals roles in adaptation to stationary phase, heat, and oxidative stress. *J Bacteriol* **192**, 2491–2502.
- Jakimowicz, P., Cheesman, M. R., Bishai, W. R., Chater, K. F., Thomson, A. J. & Buttner, M. J. (2005). Evidence that the *Streptomyces* developmental protein WhiD, a member of the WhiB family, binds a [4Fe–4S] cluster. *J Biol Chem* **280**, 8309–8315.
- Jakimowicz, D., Mouz, S., Zakrzewska-Czerwinska, Z. & Chater, K. F. (2006). Developmental control of a *parAB* promoter leads to formation of sporulation-associated ParB complexes in *Streptomyces coelicolor*. *J Bacteriol* **188**, 1710–1720.
- Jiang, H. & Kendrick, K. E. (2000). Characterization of *ssfR* and *ssgA*, two genes involved in sporulation of *Streptomyces griseus*. *J Bacteriol* **182**, 5521–5529.
- Kang, S. H., Huang, J., Lee, H. N., Hur, Y. A., Cohen, S. N. & Kim, E. S. (2007). Interspecies DNA microarray analysis identifies *wblA* as a pleiotropic down-regulator of antibiotic biosynthesis in *Streptomyces*. *J Bacteriol* **189**, 4315–4319.
- Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F. & Hopwood, D. A. (2000). *Practical Streptomyces Genetics*. Norwich: John Innes Foundation.
- Kim, T. H., Park, J. S., Kim, H. J., Kim, Y., Kim, P. & Lee, H. S. (2005). The *whcE* gene of *Corynebacterium glutamicum* is important for survival following heat and oxidative stress. *Biochem Biophys Res Commun* **337**, 757–764.
- Kim, D. W., Hesketh, A., Kim, E. S., Song, J. Y., Lee, D. H., Kim, I. S., Chater, K. F. & Lee, K. J. (2008). Complex extracellular interactions of proteases and a protease inhibitor influence multicellular development of *Streptomyces coelicolor*. *Mol Microbiol* **70**, 1180–1193.
- Kim, S. H., Lee, H. N., Kim, H. J. & Kim, E. S. (2011). Transcriptome analysis of an antibiotic downregulator mutant and synergistic actinorhodin stimulation via disruption of a precursor flux regulator in *Streptomyces coelicolor*. *Appl Environ Microbiol* **77**, 1872–1877.
- Kwak, J., Dharmatilake, A. J., Jiang, H. & Kendrick, K. E. (2001). Differential regulation of *ftsZ* transcription during septation of *Streptomyces griseus*. *J Bacteriol* **183**, 5092–5101.
- Langhorst, M. F., Reuter, A. & Stuermer, C. A. O. (2005). Scaffolding microdomains and beyond: the function of reggie/flotillin proteins. *Cell Mol Life Sci* **62**, 2228–2240.
- Lian, W., Jayapal, K. P., Charaniya, S., Mehra, S., Glod, F., Kyung, Y. S., Sherman, D. H. & Hu, W. S. (2008). Genome-wide transcriptome analysis reveals that a pleiotropic antibiotic regulator, AfsS, modulates nutritional stress response in *Streptomyces coelicolor* A3(2). *BMC Genomics* **9**, 56.
- López, D. & Kolter, R. (2010). Functional microdomains in bacterial membranes. *Genes Dev* **24**, 1893–1902.
- Molle, V., Palframan, W. J., Findlay, K. C. & Buttner, M. J. (2000). WhiD and WhiB, homologous proteins required for different stages of sporulation in *Streptomyces coelicolor* A3(2). *J Bacteriol* **182**, 1286–1295.
- Morris, R. P., Nguyen, L., Gatfield, J., Visconti, K., Nguyen, K., Schnappinger, D., Ehrt, S., Liu, Y., Heifets, L. & other authors (2005). Ancestral antibiotic resistance in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* **102**, 12200–12205.
- Nieselt, K., Battke, F., Herbig, A., Bruheim, P., Wentzel, A., Jakobsen, O. M., Sletta, H., Alam, M. T., Merlo, M. E. & other authors (2010). The dynamic architecture of the metabolic switch in *Streptomyces coelicolor*. *BMC Genomics* **11**, 10.
- Page, M. S., Chamberlin, L., Atrih, A., Foster, S. J. & Buttner, M. J. (1999). Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). *J Bacteriol* **181**, 204–211.
- Plaskitt, K. A. & Chater, K. F. (1995). Influences of developmental genes on localized glycogen deposition in colonies of a mycelial

- prokaryote, *Streptomyces coelicolor* A3(2): a possible interface between metabolism and morphogenesis. *Phil Trans Roy Soc B* **347**, 105–121.
- Raghunand, T. R. & Bishai, W. R. (2006a). Mapping essential domains of *Mycobacterium smegmatis* WhmD: insights into WhiB structure and function. *J Bacteriol* **188**, 6966–6976.
- Raghunand, T. R. & Bishai, W. R. (2006b). *Mycobacterium smegmatis* whmD and its homologue *Mycobacterium tuberculosis* whiB2 are functionally equivalent. *Microbiology* **152**, 2735–2747.
- Redenbach, M., Kieser, H. M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H. & Hopwood, D. A. (1996). A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. *Mol Microbiol* **21**, 77–96.
- Rybniiker, J., Nowag, A., van Gumpel, E., Nissen, N., Robinson, N., Plum, G. & Hartmann, P. (2010). Insights into the function of the WhiB-like protein of mycobacteriophage TM4 – a transcriptional inhibitor of WhiB2. *Mol Microbiol* **77**, 642–657.
- Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Singh, A., Guidry, L., Narasimhulu, K. V., Mai, D., Trombley, J., Redding, K. E., Giles, G. I., Lancaster, J. R., Jr & Steyn, A. J. (2007). *Mycobacterium tuberculosis* WhiB3 responds to O₂ and nitric oxide via its [4Fe–4S] cluster and is essential for nutrient starvation survival. *Proc Natl Acad Sci U S A* **104**, 11562–11567.
- Singh, A., Crossman, D. K., Mai, D., Guidry, L., Voskuil, M. I., Renfrow, M. B. & Steyn, A. J. (2009). *Mycobacterium tuberculosis* WhiB3 maintains redox homeostasis by regulating virulence lipid anabolism to modulate macrophage response. *PLoS Pathog* **5**, e1000545.
- Smith, L. J., Stapleton, M. R., Fullstone, G. J. M., Crack, J. C., Thomson, A. J., Le Brun, N. E., Hunt, D. M., Harvey, E., Adinolfi, S. & other authors (2010). *Mycobacterium tuberculosis* WhiB1 is an essential DNA-binding protein with a nitric oxide-sensitive iron-sulfur cluster. *Biochem J* **432**, 417–427.
- Soliveri, J., Vijgenboom, E., Granozzi, C., Plaskitt, K. A. & Chater, K. F. (1993). Functional and evolutionary implications of a survey of various actinomycetes for homologues of two *Streptomyces coelicolor* sporulation genes. *J Gen Microbiol* **139**, 2569–2578.
- Soliveri, J. A., Gomez, J., Bishai, W. R. & Chater, K. F. (2000). Multiple paralogous genes related to the *Streptomyces coelicolor* developmental regulatory gene *whiB* are present in *Streptomyces* and other actinomycetes. *Microbiology* **146**, 333–343.
- Steyn, A. J. C., Collins, D. M., Hondalus, M. K., Jacobs, W. R., Jr, Kawakami, R. P. & Bloom, B. R. (2002). *Mycobacterium tuberculosis* WhiB3 interacts with RpoV to affect host survival but is dispensable for *in vivo* growth. *Proc Natl Acad Sci U S A* **99**, 3147–3152.
- Takano, H., Tanaka, T., Beppu, T. & Ueda, K. (2008). Identification of *sas*, a conserved gene cluster involved in the regulation of aerial mycelium formation in *Streptomyces griseus*. *Microbiology* **154**, 3668–3675.
- Traag, B. A., Kelemen, G. H. & Van Wezel, G. P. (2004). Transcription of the sporulation gene *ssgA* is activated by the IclR-type regulator SsgR in a *whi*-independent manner in *Streptomyces coelicolor* A3(2). *Mol Microbiol* **53**, 985–1000.
- van Keulen, G., Hopwood, D. A., Dijkhuizen, L. & Sawers, R. G. (2005). Gas vesicles in actinomycetes: old buoys in novel habitats? *Trends Microbiol* **13**, 350–354.
- van Wezel, G. P., van der Meulen, J., Kawamoto, S., Luiten, R. G. M., Koerten, H. K. & Kraal, B. (2000). *ssgA* is essential for sporulation of *Streptomyces coelicolor* A3(2) and affects hyphal development by stimulating septum formation. *J Bacteriol* **182**, 5653–5662.
- Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F. & van Sinderen, D. (2007). Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiol Mol Biol Rev* **71**, 495–548.
- Willemse, J., Borst, J. W., de Waal, E., Bisseling, T. & van Wezel, G. P. (2011). Positive control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces*. *Genes Dev* **25**, 89–99.
- Willey, J. M., Willems, A., Kodani, S. & Nodwell, J. R. (2006). Morphogenetic surfactants and their role in the formation of aerial hyphae in *Streptomyces coelicolor*. *Mol Microbiol* **59**, 731–742.
- Xu, H., Chater, K. F., Deng, Z. & Tao, M. (2008). A cellulose synthase-like protein involved in hyphal tip growth and morphological differentiation in *streptomyces*. *J Bacteriol* **190**, 4971–4978.
- Xu, G., Wang, J., Wang, L., Tian, X., Yang, H., Fan, K., Yang, K. & Tan, H. (2010). “Pseudo” γ -butyrolactone receptors respond to antibiotic signals to coordinate antibiotic biosynthesis. *J Biol Chem* **285**, 27440–27448.

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