



ELSEVIER

γ -Butyrolactones: *Streptomyces* signalling molecules regulating antibiotic production and differentiation

Eriko Takano^{1,2}

Small signalling molecules called γ -butyrolactones are mainly produced by *Streptomyces* species in which they regulate antibiotic production and morphological differentiation. Their molecular mechanism of action has recently been unravelled in several streptomycetes, revealing a diverse and complex system. γ -Butyrolactones and their receptors also occur in some other Actinobacteria, suggesting that this is a general regulatory system for antibiotic production. The γ -butyrolactones bind to receptors, many of which are involved in regulation of specific antibiotic biosynthesis clusters. The importance of understanding how secondary metabolites are regulated and how environmental and physiological signals are sensed highlights the relevance of studying this system.

Addresses

¹ Mikrobiologie/Biotechnologie, Eberhard-Karls-Universität Tübingen, Elfriede-Aulhorn Str 6, 72076 Tübingen, Germany

² Groningen Biomolecular Sciences and Biotechnology Institute, Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Corresponding author: Takano, Eriko (e.takano@rug.nl)

Current Opinion in Microbiology 2006, **9**:287–294

This review comes from a themed issue on Ecology and industrial microbiology Edited by Arnold Demain and Lubbert Dijkhuizen

Available online 3rd May 2006

1369-5274/\$ – see front matter

© 2006 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.mib.2006.04.003

Introduction

Bacterial cell-to-cell communication by small signalling molecules, in particular *N*-acyl-homoserine lactones (AHLs) in proteobacteria, has been studied intensively over the past decade [1^{**}–3^{**}]. However, the first signalling molecules to be identified were the γ -butyrolactones from *Streptomyces* in the 1960s, and these also have recently seen a flowering of interest [4^{**},5–7].

Streptomyces is a genus of Gram-positive soil bacteria with complex morphological development. It produces more than 70% of commercially available antibiotics [8]. Pioneer work by Khokhlov [9] identified a γ -butyrolactone from *Streptomyces griseus* (also known as autoregulatory factor or A-factor) that induced streptomycin production and sporulation. The structures of fourteen 2,3-disubstituted γ -butyrolactones that are from seven *Streptomyces*

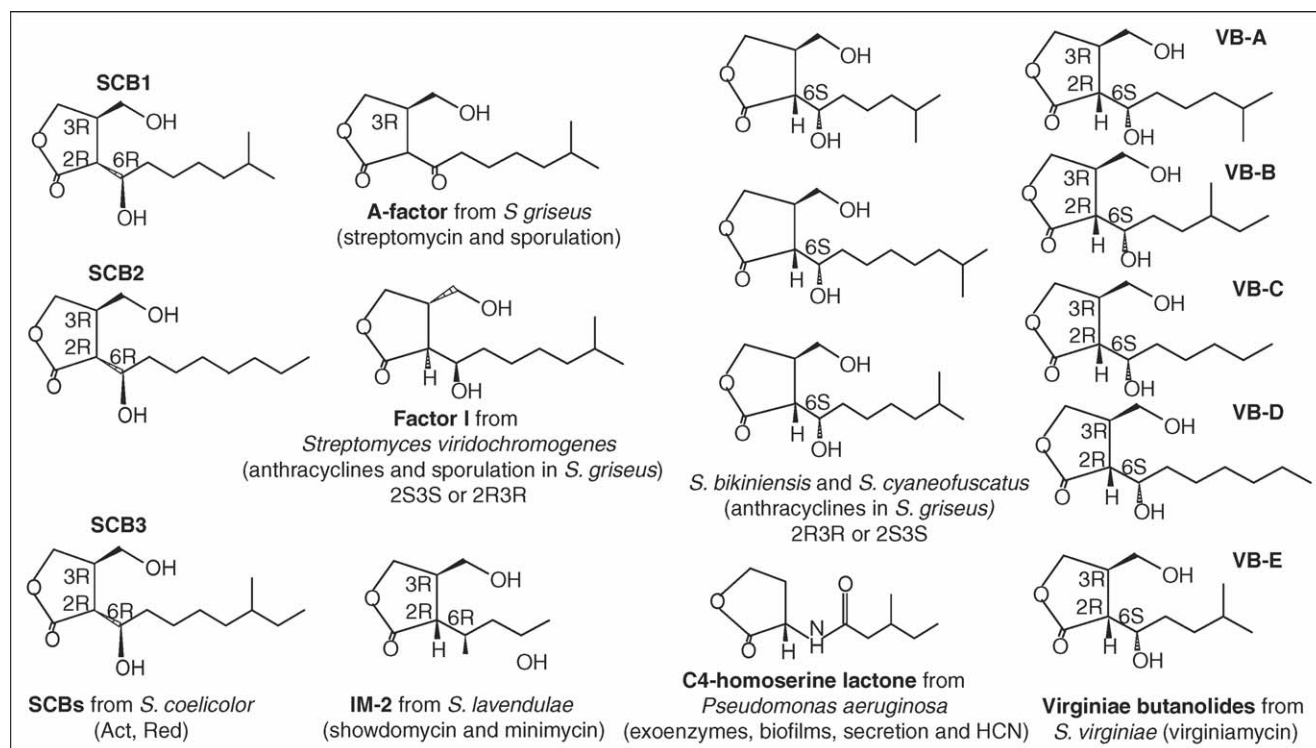
species have been determined so far, and they differ in length, branching and the stereochemistry of their fatty acid side-chain (Figure 1) [5–7]. All of these factors regulate the production of antibiotics, are effective in nano Molar concentrations and in some cases they regulate differentiation. The γ -butyrolactones bind to cytoplasmic receptor proteins (e.g. ArpA in *S. griseus*) and inhibit their binding to specific DNA targets. Most of these receptor proteins act as repressors, so that binding to γ -butyrolactones induces expression of the target genes. Each receptor protein is highly specific for its cognate γ -butyrolactone [4^{**},5–7]. Biosynthesis of γ -butyrolactones is not well understood, but seems to require a member of a protein family, the archetype of which is AfsA in *S. griseus*. The chemical structure of γ -butyrolactones is similar to that of AHLs except for the carbon side-chain (Figure 1). However, the γ -butyrolactone receptors do not bind to AHL, and AHL receptors do not bind to γ -butyrolactones (E Takano, unpublished); this is also confirmed by the low similarity of both signalling molecule receptors. The functions of the signalling molecules also seems to differ as AHLs have very diverse properties [1^{**}–3^{**}], whereas the γ -butyrolactones mainly regulate the production of antibiotics and differentiation [4^{**},5–7]. Furthermore, the synthesis of the signalling molecule also differs as LuxI, the AHL synthase, is not similar to AfsA.

There are many reviews on the A-factor system from *S. griseus* and the virginiae butanolide (VB) system from *Streptomyces virginiae* [4^{**},5–7]. Here, I focus mainly on new insights into different aspects of the γ -butyrolactone regulatory system, especially in *Streptomyces coelicolor*, and demonstrate its diversity.

The *S. coelicolor* butanolide system

The structures of *S. coelicolor* γ -butyrolactones active in the A-factor bioassay were proposed in the 1980s [10]. Recently, three γ -butyrolactones have been determined, and more are anticipated to be determined from high-performance liquid chromatography (HPLC) analysis and bioassays [11] (E Takano, unpublished) (Figure 1). The most abundant is *S. coelicolor* butanolide 1 (SCB1), reported previously to stimulate actinorhodin (Act) and undecylprodigiosin (Red) production. The SCBs are only active in a narrow concentration range of 0.25–0.5 μ M, suggesting strictly controlled expression and production of these small molecules. The SCBs are more stable than A-factor, possibly because of the hydroxyl group at C6, and can still be found more than 12 h into stationary phase. Degradation of SCBs has been observed only in some rich media (E Takano, unpublished). Whether this

Figure 1



Chemical structures of γ -butyrolactones from *Streptomyces* and C4-homoserine lactone from *Pseudomonas aeruginosa*. The name of the signalling molecules appear in bold, and the antibiotic that it effects or its other functions are shown in brackets.

is as a result of active degradation or of a pH change in the cells that causes the lactone ring to open is not certain. SCBs are produced at late transition phase when expression of *scbA*, a gene involved in SCB synthesis (*afsA* homologue), is induced rather than accumulating from early exponential phase as in the A-factor system. Thus the *scbA* deletion mutant does not produce any of the γ -butyrolactones which have antibiotic-stimulatory activity [12]. Along with *scbA*, an *arpA*-like γ -butyrolactone receptor gene, *scbR*, has been characterised. ScbR binds to its own promoter and to that of the adjacent diverging *scbA*, thus regulating production of SCBs [12]. Corresponding receptor protein targets have not been clearly identified in other systems, although homologues of both genes exist in all of them. Microarray analysis revealed that ScbR also represses the pathway-specific activator gene, *kasO*, which activates a biosynthetic gene cluster (*kas*, located next to *scbR*) that codes for synthesis of an unknown polyketide [13^{••}]. In the *scbR* mutant, expression of most of the genes in the *kas* cluster was greatly increased. The DNA sequence of two ScbR target sites, shown to be repressor sites, was completely conserved [13^{••}]. However the proposed consensus target sequences for γ -butyrolactone receptors all have considerable variability [5]. From the ScbR binding consensus sequence, only one other conserved sequence was identified upstream of *orfB*, two genes away from *scbR*. The role

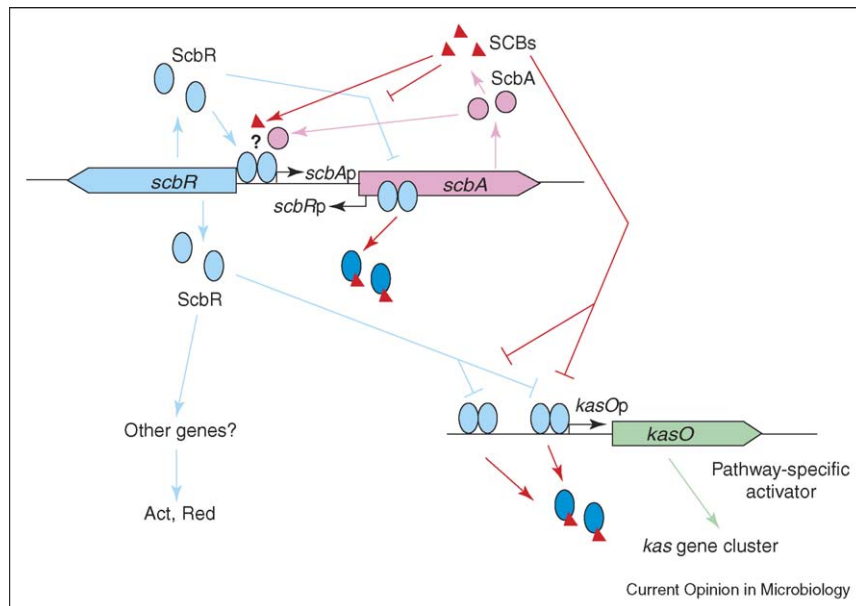
of this gene is still under investigation. The SCB system is summarised as a model in Figure 2.

An alternative method for γ -butyrolactone determination, and γ -butyrolactones from non-streptomycetes

Structural determination of a new γ -butyrolactone was last reported in 2001 [11]. This is surprising as there are many reports of γ -butyrolactone receptors in the last three years (see below). The very small amount of γ -butyrolactones produced in *Streptomyces* cultures and the limited ability to detect the signalling molecules by bioassays might be the reason for the paucity of more structural determinations.

Recently, Yang *et al.* [14^{••}] reported an alternative detection system using ScbR, the receptor protein and electro-spray tandem mass spectrometry (ESI-MS/MS). This method does not require large amounts of cultures and might be useful for those strains where the γ -butyrolactone receptors have already been identified. However, with this method, only SCB1 was identified from *S. coelicolor*, even though at least two other γ -butyrolactones also bind to ScbR (E Takano, unpublished). Possibly, SCB1 was the most abundant γ -butyrolactone, and this might explain why the others were not identified. γ -Butyrolactones were also identified from non-*Streptomyces* actinomycetes that produce commercially important

Figure 2



Schematic model of the SCB system in *S. coelicolor*. ScbR (light-blue ovals) binds as a dimer to 4 targets sites. One repressor site is in front of its own promoter (*scbRp*) and two other repressor sites are in front of the *kasO* (pathway-specific activator) promoter (*kasOp*). In all cases, the γ -butyrolactones (SCBs; red triangles) bind to ScbR (deep-blue ovals with triangle) and relieves the repression. ScbA (pink circles) is involved in production of SCBs and also is required along with SCBs and ScbR for its own expression (*scbAp*, the *scbA* promoter). This precise mechanism is under investigation (shown as '?'). ScbR might regulate other genes which could in turn regulate the production of Act and Red. KasO actively regulates the transcription of the *kas* gene cluster. Arrows denote activation and lines with a bar denote repression.

antibiotics [15]. *Amycolatopsis mediterranei* that produces rifamycin, and *Actinoplanes teichomyceticus*, a teicoplanin producer, were both found to produce γ -butyrolactones using a bioassay for IM-2 of *Streptomyces lavendulae* and VB of *S. virginiae*, respectively. In the same report, γ -butyrolactone receptor proteins were also identified from *A. mediterranei*, *A. teichomyceticus*, and *Micromonospora echinospora*, a gentamicin producer, binding to IM-2, VB or SCB1, and SCB1 respectively. This report suggests that γ -butyrolactone signalling systems are widespread regulators of antibiotic production in actinomycetes.

γ -Butyrolactone synthesis: are AfsA homologues enzymes or regulators?

There is an unresolved contradiction concerning the function of AfsA and its *S. virginiae* homologue BarX. AfsA, a putative A-factor biosynthetic gene, has been reported to condense a glycerol derivative and a β -keto acid (derived from fatty acid biosynthesis) to produce γ -butyrolactones [16], whereas BarX has been reported to stabilise receptor–DNA binding, suggesting a regulatory function [17]. There are several reports of AfsA homologues (Table 1), but no clear function has been assigned to any for these and *in vitro* synthesis of γ -butyrolactones has not yet been found. Furthermore, no homologues have been identified in the DNA or protein databases for this family of proteins, apart from one partial homologue (see below).

All *afsA*-family genes — apart from *afsA* itself — are located either very close to, or in, antibiotic biosynthetic clusters, and many are either adjacent or close to, and sometimes divergent from, their respective γ -butyrolactone receptor gene (Table 1).

This is the case for the gene-product of *scbA*, the AfsA homologue in *S. coelicolor*, which is located near a recently identified polyketide biosynthesis cluster [13•] and is divergent from *scbR* [12]. However, ScbA might possess both regulatory and enzymatic functions. By microarray analysis, many primary metabolic and regulatory genes were identified which are differentially expressed when the *scbA* mutant was compared to the wild type in early exponential phase (E Takano, unpublished). Furthermore, mutagenesis of a conserved amino acid residue of ScbA, selected using computer-assisted active-site predictions, eliminated γ -butyrolactone production (E Takano, unpublished). It is intriguing that a gene product from *Gloeobacter violaceus* PCC 7421 (a cyanobacterium), which is annotated as a polyketide synthase because most of the domains in the protein resemble acyl transferase domains and phosphopantetheine attachment sites, contains an Afs domain (Pfam03756), indicating that the protein has an enzymatic role. Although a pathway for γ -butyrolactone biosynthesis was suggested in 1992 [18], the only further

Table 1

ScbA homologues from the EMBL database

Protein names	Organism	Amino acid identity ^a (%)	Receptor gene location ^b	Antibiotic cluster ^c	References and year
ScbA	<i>S. coelicolor</i>	100	Divergent	Kas	[12] 2001
AfsA	<i>S. griseus</i>	66	100 kb away		[16] 1997
JadW1	<i>S. venezuelae</i>	45	Two genes away, divergent	Jadomycin	[41] 2003
BarX	<i>S. virginiae</i>	43	Divergent	Virginiamycin	[17] 2000
Orf85	<i>S. rochei</i> (plasmid pSLA2-L)	37	Two genes away, divergent		[27] 2003
NcsR1	<i>S. carzinostaticus</i> ATCC 15944	41	Adjacent	Neocarzino-staten	[28] 2005
FarX	<i>S. lavendulae</i>	40	Adjacent		[42] 1997
Orf2	<i>S. natalensis</i>	36	Divergent	Natamycin	[43] 2005
AvaA	<i>S. avermitilis</i> MA-4680	32	Homologue on both sides		[21] 2003
MmfL	<i>S. coelicolor</i> (plasmid SCP1)	29	Divergent	Methylenomycin	[44] 2004

^a Amino acid identity with ScbA from blast searches performed at NCBI (<http://www.ncbi.nlm.nih.gov>).

^b Location of the cognate γ -butyrolactone receptors in relation to the ScbA homologues.

^c Name of antibiotic biosynthesis gene cluster where the ScbA homologues are located. Where nothing is indicated, the results are unknown.

report of a proven γ -butyrolactone biosynthetic step involves BarS1, which catalyses the last reduction in C6 of VB biosynthesis [6,19].

γ -Butyrolactone receptors

Are they pathway-specific regulators?

The enormous increase in the number of γ -butyrolactone receptor homologues that have been identified in the past three years (Table 2) reflects the fact that the nucleotide sequences of many gene clusters that encode production of secondary metabolites have recently become available, including those in the complete genome sequences of *S. coelicolor* [20] and *Streptomyces avermitilis* [21]. At least 22 out of 33 genes encoding homologues of γ -butyrolactone receptors are located close to antibiotic biosynthesis genes and/or have been shown to regulate antibiotic production [13^{**}]. Thus, it seems that γ -butyrolactones are strongly associated with the regulation of antibiotic production and that most of the γ -butyrolactone receptors could be pathway-specific. It is interesting that, for the first time, a receptor protein from a non-streptomycete has been cloned and disrupted with a positive effect on antibiotic production [22^{**}].

Several mutagenesis studies of γ -butyrolactone receptors have been reported (Table 2). In most cases, deletion of the receptor caused overproduction of an antibiotic, as in the paradigm *S. griseus* system. However, for some receptor genes, antibiotic production was delayed or abolished [23–25]. In *S. coelicolor*, it was initially thought that deletion of the receptor genes delayed synthesis of Act and Red, but the recent finding that the *scbR*-linked *kas* cluster was overexpressed in the *scbR* mutant [13^{**}] suggests that the γ -butyrolactone receptor primarily represses biosynthesis of the *kas* product, and that production of other antibiotics might then be indirectly affected by abundance of precursors, or by other unknown effects on the expression of the other antibiotic biosynthesis genes.

The role of multiple receptor genes

In many streptomycetes multiple γ -butyrolactone receptor homologues exist, and in some they all are located in antibiotic biosynthesis gene clusters [26^{*},27,28]. The best characterised examples of such systems are the VB system [29–31] and the tylosin system from *Streptomyces fradie* [32–34]. In both cases, studies with mutants showed that a main receptor represses other homologues, forming a regulatory cascade leading to antibiotic production. However, in neither case is there biochemical evidence showing direct regulation by these main receptors on the homologues.

In *S. coelicolor*, three receptors, other than ScbR, have been identified, as well as ScbR2. Previous reports indicate that *cprA* and *cprB* mutants show that when mutated separately, the resulting mutants have the opposite phenotype in both antibiotic production and sporulation, i.e. the *cprA* mutant has reduced antibiotic production and delayed sporulation, whereas the *cprB* mutant overproduces antibiotics and sporulates earlier. Therefore, CprA is an activator for both antibiotic production and sporulation whereas CprB is a repressor. [35]. We independently mutated *cprA* and *cprB* to find that there was no obvious phenotype in these mutants. However, a *cprAB* double deletion mutant was unable to sporulate (E Takano, unpublished). These differences in the mutant phenotypes in different laboratories might be as a result of the different parent strains that were used for the mutagenesis. The roles of CprA, CprB and ScbR2 still need to be clarified: preliminary evidence suggests that ScbR2 is involved in regulation of the Kas cluster, of which *scbR2* forms a part; *cprB* is located two genes upstream of the geosmin biosynthesis gene cluster [20,36], but its effect on geosmin production has not yet been determined; and *cprA* is not located close to a known antibiotic biosynthesis gene cluster. Unlike the VB system and that of tylosin, ScbR does not directly regulate expression of the other receptor homologues [13] (E Takano,

Table 2

List of ScbR homologues from the EMBL data base

Gene names/ acc numbers	Organism	Amino acid identity ^a (%)	Antibiotic production ^b	Sporu- lation ^c	Antibiotic gene cluster ^d	References and year
ScbR/NP-630365	<i>S. coelicolor</i>	100	(-): Act, Red +: Kas	±	Kas	[12] 2001
FarA/BAA21859	<i>S. lavedulae</i>	57	+: Nucleoside, +: Blue pigment +: D-cycloserine	±		[45]1997
Orf82/NP-851504	<i>S. rochei</i> (plasmid pSLA2-L)	51				[27] 2003
AlpZ/AAR30170	<i>S. ambofaciens</i>	48			Alpomycin	[26*] 2004
BarA/A57507	<i>S. virginiae</i>	47	+: Virginiamycin	±	Virginiamycin	[29] 1997
AvaR/NP-824882	<i>S. avermitilis</i> MA-4680	47				
TarA/AAF06961	<i>S. tendae</i>	48	(-): Nikkomycin			[23] 2001
SabR/AY256849.1	<i>S. ansochromogenes</i>	48	(-): Nikkomycin,	+		[25] 2003
ScaR/BAC66444.1	<i>S. clavuligerus</i>	48				[37] 2004
Brp/CAH55691.1			+: Clavulanic acid +: Cephamycin	±		[38**] 2005
SpbR/AAK07686	<i>S. pristinaespiralis</i>	48	-: Pristinamycin	±	Pristinamycin	[24] 2002
Sng/AAx97699.1	<i>S. natalensis</i>	44	+: Natamycin	+	Natamycin	[43] 2005
TyIP/T44586	<i>S. fradiae</i>	45	+: Tylosin	±	Tylosin	[32] 1999
ArpA/BAA08617	<i>S. griseus</i>	41	+: Streptomycin	-		[46] 1995
KsbA/BAD20233.1	<i>Kitasatospora setae</i>	38	+: Bafilomycin	±		[22**] 2005
NcsR2/AAM78022	<i>S. carzinostaticus</i> ATCC15944	39			Neocarzinostaten	[28] 2005
AAR90230	<i>Rhodococcus</i> sp. DK17 34 (plasmid PKD3)					
SAV3702/NP-824879	<i>S. avermitilis</i> MA-4680	33				
BarB/BAA23612	<i>S. virginiae</i>	37	+: Virginiamycin	±	Virginiamycin	[29] 1997
Orf79/NP-851501	<i>S. rochei</i> (plasmid pSLA2-L)	34				[27] 2003
MmfR/NP-639852	<i>S. coelicolor</i> (plasmid SCP1)	30			Methylemomycin	[44] 2004
ZP00378009.1	<i>Brevibacterium linens</i> BL2	29				
Aur1R/AAx57186	<i>S. aureofaciens</i>	32				[47] 2005
ApIW/AAR30167	<i>S. ambofaciens</i>	31			Alpomycin	[26*] 2004
TyIQ/T44588	<i>S. fradiae</i>	35	+: Tylosin		Tylosin	[32] 1999
SCO6286/NP-630384	<i>S. coelicolor</i>	32				
Orf74/NP-851496	<i>S. rochei</i> (plasmid pSLA2-L)	34				[27] 2003
CprB/NP-630180	<i>S. coelicolor</i>	32				[35] 1998
SeaR/BAD89597.1	<i>Saccaropolyspora erythraea</i>	34				
MAP0928/NP-959902	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> str. k10	32				
SCO6323/NP-630417	<i>S. coelicolor</i>	29				
CprA/NP_630409	<i>S. coelicolor</i>	32				[35] 1998
SAV2270/NP823444.1	<i>S. avermitilis</i> MA-4680	31				
Ava2488/YP322998.1	<i>Anabaena variabilis</i> ATCC29413	30				
JadR2/AAB36583	<i>S. venezuelae</i>	34			Jadomycin	[41] 2003
SAV2268/NP-823446	<i>S. avermitilis</i> MA-4680	31				
PBD2.026/NP-898641	<i>Rhodococcus erythropolis</i>	28				
BAD59728.1	<i>Nocardia farcinica</i> FM10152	30				
Alr4567/NP-488607	<i>Nostoc</i> sp. PCC 7120	31				
BAD55455.1	<i>Nocardia farcinical</i> FM10152	28				
BAE460301.1	<i>Rhodococcus erythropolis</i>	36				
AAR90151.1	<i>Rhodococcus</i> sp. DK17	32				

^a Amino acid identity with ScbR from BLAST searches performed at National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

^b Antibiotic production phenotype of the receptor mutant; +, increased production; -, decreased production; (-), delayed production; not indicated, unknown.

^c Sporulation phenotype of the receptor mutant; +, increased sporulation; -, no sporulation; ±, same as parent; not indicated, unknown.

^d Name of the antibiotic biosynthesis gene cluster which it is close to or part of; not indicated, unknown.

unpublished); this is intriguing, what does regulate expression of these homologues? Understanding this will, in turn, aid in understanding of the role of these homologues in *S. coelicolor*.

Multiple proteins can bind to the γ-butyrolactone receptor binding site

A γ-butyrolactone receptor homologue from *S. clavuligerus* was identified in two different laboratories (this gene

has two different names, *scaR* and *brp*) [37,38**]. Interestingly, the identified target sequence for the receptor that is upstream of the pathway-specific regulator has been shown to bind to another protein [38**]. This report is a first example of multiple proteins binding to a γ -butyrolactone-receptor target sequence. The determination of the second protein is of extreme interest.

Crystal structure of the γ -butyrolactone receptor

The first crystal structure of a γ -butyrolactone receptor, CprB from *S. coelicolor*, was determined by Natsume *et al.* [39*]. Two forms of the structure were obtained, both dimers, and the overall structure resembles that of the TetR family. The large cavity found in the regulatory domain is probably the γ -butyrolactone binding pocket, with conserved amino acid residues within the cavity. It is thought that the conformation of the protein changes with binding of the γ -butyrolactones, causing the DNA-binding domain to relocate, and CprB to dissociate from the DNA. This crystal structure will aid in understanding the structural similarity of the γ -butyrolactone receptor homologues. However, it is worth noting that CprB has not biochemically been shown to bind to a *S. coelicolor* DNA sequence or a γ -butyrolactone. Further crystal studies of receptors known to bind to DNA with and without cognate γ -butyrolactones will be of interest.

Conclusions

γ -Butyrolactone signalling has been dominated by the A-factor system in *S. griseus* because it was the first signalling cascade into antibiotic production to be reported [5]. Recently, as many reports on γ -butyrolactone systems in different actinomycetes have appeared, the diversity and the complexity of the γ -butyrolactone signalling system are becoming clear [40].

The detailed mechanisms of the γ -butyrolactone signalling system — these are synthesis, receptor specificity to DNA sequences, multiple signals and receptors, degradation, the effect of γ -butyrolactone on primary metabolism and antibiotic production, and signalling cascades — are just a few of the aspects that need further detailed study. We have only seen the tip of this iceberg. We need to fully understand the role of these small signalling molecules, whether it is to regulate and coordinate antibiotic production, to sense environmental and physiological signals, or to communicate or coordinate with other bacteria or within the multiple compartments of the same organism. With increasing interest — as seen by the number of publications on the topic, boosted by the genome sequences of *S. coelicolor*, *S. avermitilis* and other streptomycetes — many of these questions will surely be answered in the near future.

Acknowledgements

Thanks to David Hopwood, Keith Chater, Bertrand Aigle and Marco Gottelt for comments on the manuscript. Also thanks to the members of my group and the Microbiology/Biotechnology group in the University

of Tuebingen. The work was funded by Deutsche Forschungsgemeinschaft (TA428/1-1, TA428/2-1) and also by EUFP6 Actinogen.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Camilli A, Bassler BL: **Bacterial small-molecule signaling pathways**. *Science* 2006, **311**:1113-1116.
See note for [3**]
2. Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR: **Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria**. *Nat Rev Microbiol* 2005, **3**:383-396.
See note for [3**]
3. Venturi V: **Regulation of quorum sensing in Pseudomonas**. *FEMS Microbiol Rev* 2006, **30**:274-291.
Reviews [1**–3**] are all up-to-date views of the signalling molecules in Proteobacteria
4. Ohnishi Y, Yamazaki H, Kato JY, Tomono A, Horinouchi S: **AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in Streptomyces griseus**. *Biosci Biotechnol Biochem* 2005, **69**:431-439.
The most recent review on the *S. griseus* A-factor system that focuses on AdpA, the central activator. This includes the AdpA regulon and the DNA binding mechanism.
5. Horinouchi S: **A microbial hormone, A-factor, as a master switch for morphological differentiation and secondary metabolism in Streptomyces griseus**. *Front Biosci* 2002, **7**:d2045-d2057.
6. Nihira T: **Virginiamycin: biosynthetic pathway and its regulation, with special emphasis on the genetic aspects and autoregulation-dependent regulation**. In *Microbial Secondary Metabolites: Biosynthesis, Genetics and Regulation*. Edited by Fierro F, Martin JF. Kerala, India: Research Signpost; 2002:99-119.
7. Yamada Y: **In Autoregulatory factors and regulation of antibiotic production in Streptomyces: in microbial signalling and communication**. Edited by England R, Hobbs G, Bainton N, McRoberts DL. Cambridge, UK: Society for General Microbiology, Cambridge University Press; 1999:177-196.
8. Weber T, Welzel K, Pelzer S, Vente A, Wohlleben W: **Exploiting the genetic potential of polyketide producing streptomycetes**. *J Biotechnol* 2003, **106**:221-232 Review.
9. Khokhlov AS, Tovarova II, Borisova LN, Pliner SA, Shevchenko LA, Kornitskaya EYa, Ivkina NS, Rapoport IA: **The A-factor, responsible for streptomycin biosynthesis by mutant strains of Actinomyces streptomycini**. *Dokl Akad Nauk SSSR* 1967, **177**:232-235.
10. Anisova LN, Bliinova IN, Efremenkova OV, Koz'min YuP, Onoprienko VV, Smirnova GM, Khokhlov AS: **Development regulators in Streptomyces coelicolor A3(2)**. In *Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya*. MM Shemyakin Institute of Bioorganic Chemistry, Moscow; 1984. 1:98-108.
11. Takano E, Nihira T, Hara Y, Jones JJ, Gershter CJL, Yamada Y, Bibb MJ: **Purification and structural determination of SCB1, a γ -butyrolactone that elicits antibiotic production in Streptomyces coelicolor A3(2)**. *J Biol Chem* 2000, **275**:11010-11016.
12. Takano E, Chakraborty R, Nihira T, Yamada Y, Bibb MJ: **A complex role for the γ -butyrolactone SCB1 in regulating antibiotic production in Streptomyces coelicolor A3(2)**. *Mol Microbiol* 2001, **41**:1015-1028.
13. Takano E, Kinoshita H, Mersinias V, Bucca G, Hotchkiss G, Nihira T, Smith C, Bibb M, Wohlleben W, Chater KF: **A bacterial hormone (the SCB1 extracellular signalling system) directly controls an antibiotic pathway-specific regulator in the cryptic**

type I polyketide biosynthetic cluster of *Streptomyces coelicolor* A3(2). *Mol Microbiol* 2005, **56**:465-479.

This study contains the most recent data on the *S. coelicolor* SCB regulon. Data shows that the γ -butyrolactone receptor directly regulates a pathway-specific regulatory gene for an antibiotic biosynthesis gene cluster.

14. Yang YH, Joo HS, Lee K, Liou KK, Lee HC, Sohng JK, Kim BG:
•• **Novel method for detection of butanolides in *Streptomyces coelicolor* culture broth, using a His-tagged receptor (ScbR) and mass spectrometry.** *Appl Environ Microbiol* 2005, **71**:5050-5055.

This study demonstrates an alternative method for γ -butyrolactone detection using the receptor protein and ESI-MS/MS.

15. Choi SU, Lee CK, Hwang YI, Kinoshita H, Nihira T: **γ -butyrolactone autoregulators and receptor proteins in non-*Streptomyces* actinomycetes producing commercially important secondary metabolites.** *Arch Microbiol* 2003, **180**:303-307.

16. Ando N, Matsumori N, Sakuda S, Beppu T, Horinouchi S: **Involvement of *afsA* in A-factor biosynthesis as a key enzyme.** *J Antibiot (Tokyo)* 1997, **50**:847-852.

17. Kawachi R, Akashi T, Kamitani Y, Sy A, Wangchaisoonthorn U, Nihira T, Yamada Y: **Identification of an *AfsA* homologue (*BarX*) from *Streptomyces virginiae* as a pleiotropic regulator controlling autoregulator biosynthesis, virginiamycin biosynthesis and virginiamycin M1 resistance.** *Mol Microbiol* 2000, **36**:302-313.

18. Sakuda S, Higashi A, Tanaka S, Nihira T, Yamada Y: **Biosynthesis of virginiae butanolide A a butyrolactone autoregulator from streptomycetes.** *J Am Chem Soc* 1992, **114**:663-668.

19. Shikura N, Yamamura J, Nihira T: ***barS1*, a gene for biosynthesis of a γ -butyrolactone autoregulator, a microbial signaling molecule eliciting antibiotic production in *Streptomyces* species.** *J Bacteriol* 2002, **184**:5151-5157.

20. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D *et al.*: **Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2).** *Nature* 2002, **417**:141-147.

21. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S: **Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*.** *Nat Biotechnol* 2003, **21**:526-531.

22. Choi SU, Lee CK, Hwang YI, Kinoshita H, Nihira T: **Cloning and functional analysis by gene disruption of a gene encoding a γ -butyrolactone autoregulator receptor from *Kitasatospora setae*.** *J Bacteriol* 2004, **186**:3423-3430.

First report of cloning and disruption of a non-*Streptomyces* γ -butyrolactone receptor.

23. Engel P, Scharfenstein LL, Dyer JM, Cary JW: **Disruption of a gene encoding a putative gamma-butyrolactone-binding protein in streptomycetes *tendae* affects nikkomycin production.** *Appl Microbiol Biotechnol* 2001, **56**:414-419.

24. Folcher M, Gaillard H, Nguyen LT, Nguyen KT, Lacroix P, Bamas-Jacques N, Rinkel M, Thompson CJ: **Pleiotropic functions of a *Streptomyces pristinaespiralis* autoregulator receptor in development, antibiotic biosynthesis, and expression of a superoxide dismutase.** *J Biol Chem* 2001, **276**:44297-44306.

25. Li W, Liu G, Tan H: **Disruption of *sabR* affects nikkomycin biosynthesis and morphogenesis in *Streptomyces ansochromogenes*.** *Biotechnol Lett* 2003, **25**:1491-1497.

26. Pang X, Aigle B, Girardet JM, Manganot S, Pernodet JL, Decaris B, Leblond P: **Functional angucycline-like antibiotic gene cluster in the terminal inverted repeats of the *Streptomyces ambofaciens* linear chromosome.** *Antimicrob Agents Chemother* 2004, **48**:575-588.

The authors describe an interesting antibiotic biosynthesis cluster, which has five regulatory genes and produces two different antibiotics, found on both terminal ends of the linear chromosome of *Streptomyces ambofaciens*.

27. Mochizuki S, Hiratsu K, Suwa M, Ishii T, Sugino F, Yamada K, Kinashi H: **The large linear plasmid pSLA2-L of *Streptomyces rochei* has an unusually condensed gene organization for secondary metabolism.** *Mol Microbiol* 2003, **48**:1501-1510.

28. Liu W, Nonaka K, Nie L, Zhang J, Christenson SD, Bae J, Van Lanen SG, Zazopoulos E, Farnet CM, Yang CF, Shen B: **The neocarzinostatin biosynthetic gene cluster from *Streptomyces carzinostaticus* ATCC 15944 involving two iterative type I polyketide synthases.** *Chem Biol* 2005, **12**:293-302.

29. Kinoshita H, Ipposhi H, Okamoto S, Nakano H, Nihira T, Yamada Y: **Butyrolactone autoregulator receptor protein (*BarA*) as a transcriptional regulator in *Streptomyces virginiae*.** *J Bacteriol* 1997, **179**:6986-6993.

30. Nakano H, Takehara E, Nihira T, Yamada Y: **Gene replacement analysis of the *Streptomyces virginiae barA* gene encoding the butyrolactone autoregulator receptor reveals that *BarA* acts as a repressor in virginiamycin biosynthesis.** *J Bacteriol* 1998, **180**:3317-3322.

31. Matsuno K, Yamada Y, Lee CK, Nihira T: **Identification by gene deletion analysis of *barB* as a negative regulator controlling an early process of virginiamycin biosynthesis in *Streptomyces virginiae*.** *Arch Microbiol* 2004, **181**:52-59.

32. Bate N, Butler AR, Gandecha AR, Cundliffe E: **Multiple regulatory genes in the tylosin biosynthetic cluster of *Streptomyces fradiae*.** *Chem Biol* 1999, **6**:617-624.

33. Stratigopoulos G, Gandecha AR, Cundliffe E: **Regulation of tylosin production and morphological differentiation in *Streptomyces fradiae* by *TyIP*, a deduced γ -butyrolactone receptor.** *Mol Microbiol* 2002, **45**:735-744.

34. Stratigopoulos G, Cundliffe E: **Expression analysis of the tylosin biosynthetic gene cluster: pivotal regulatory role of the *tylQ* product.** *Chem Biol* 2002, **9**:71-78.

35. Onaka H, Nakagawa T, Horinouchi S: **Involvement of two A-factor receptor homologues in *Streptomyces coelicolor* A3(2) in the regulation of secondary metabolism and morphogenesis.** *Mol Microbiol* 1998, **28**:743-753.

36. Gust B, Challis GL, Fowler K, Kieser T, Chater KF: **PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin.** *Proc Natl Acad Sci USA* 2003, **100**:1541-1546.

37. Kim HS, Lee YJ, Lee CK, Choi SU, Yeo SH, Hwang YI, Yu TS, Kinoshita H, Nihira T: **Cloning and characterization of a gene encoding the gamma-butyrolactone autoregulator receptor from *Streptomyces clavuligerus*.** *Arch Microbiol* 2004, **182**:44-50.

38. Santamarta I, Perez-Redondo R, Lorenzana LM, Martin JF, Liras P: **Different proteins bind to the butyrolactone receptor protein ARE sequence located upstream of the regulatory *ccaR* gene of *Streptomyces clavuligerus*.** *Mol Microbiol* 2005, **56**:824-835.

This paper contains recent findings of another protein which may bind to the γ -butyrolactone receptor binding sequence.

39. Natsume R, Ohnishi Y, Senda T, Horinouchi S: **Crystal structure of a γ -butyrolactone autoregulator receptor protein in *Streptomyces coelicolor* A3(2).** *J Mol Biol* 2004, **336**:409-419.

This study describes the first crystal structure of a γ -butyrolactone receptor.

40. Chater KF, Horinouchi S: **Signalling early developmental events in two highly diverged *Streptomyces* species.** *Mol Microbiol* 2003, **48**:9-15.

41. Wang L, Vining LC: **Control of growth, secondary metabolism and sporulation in *Streptomyces venezuelae* ISP5230 by *jadW₁*, a member of the *afsA* family of γ -butyrolactone regulatory genes.** *Microbiology* 2003, **149**:1991-2004.

42. Waki M, Nihira T, Yamada Y: **Cloning and characterization of the gene (*farA*) encoding the receptor for an extracellular regulatory factor (*IM-2*) from *Streptomyces* sp. strain FRI-5.** *J Bacteriol* 1997, **179**:5131-5137.

43. Lee KM, Lee CK, Choi SU, Park HR, Kitani S, Nihira T, Hwang YI: **Cloning and in vivo functional analysis by disruption of a gene encoding the gamma-butyrolactone autoregulator receptor from *Streptomyces natalensis*.** *Arch Microbiol* 2005, **184**:249-257.

44. Bentley SD, Brown S, Murphy LD, Harris DE, Quail MA, Parkhill J, Barrell BG, McCormick JR, Santamaria RI, Losick R *et al.*: **SCP1, a 356 023 bp linear plasmid adapted to the ecology and developmental biology of its host, *Streptomyces coelicolor* A3(2).** *Mol Microbiol* 2004, **51**:1615-1628.
45. Kitani S, Yamada Y, Nihira T: **Gene replacement analysis of the butyrolactone autoregulator receptor (FarA) reveals that FarA acts as a novel regulator in secondary metabolism of *Streptomyces lavendulae* FRI-5.** *J Bacteriol* 2001, **183**:4357-4363.
46. Onaka H, Ando N, Nihara T, Yamada Y, Beppu T, Horinouchi S: **Cloning and characterization of the A-factor receptor gene from *Streptomyces griseus*.** *J Bacteriol* 1995, **177**:6083-6092.
47. Novakova R, Homerova D, Feckova L, Kormanec J: **Characterization of a regulatory gene essential for the production of the angucycline-like polyketide antibiotic auricin in *Streptomyces aureofaciens* CCM 3239.** *Microbiology* 2005, **151**:2693-2706.