**SGM
SPECIAL
LECTURE****The regulation of antibiotic production in
Streptomyces coelicolor A3(2)**

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**1995 Colworth Prize
Lecture**

(Delivered at the Golden Jubilee
Meeting of the Society for
General Microbiology, 3 April
1995)

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Keywords: *Streptomyces*, antibiotics, ppGpp, σ factors, γ -butyrolactones

**Streptomyces as producers of diverse
antibiotics**

Members of the genus *Streptomyces* belong to the family *Actinomycetales*, a division of the Gram-positive bacteria characterized by a high genomic G+C content (mean 74 mol %) (Stackebrandt & Woese, 1981; Goodfellow & Cross, 1984; Fox & Stackebrandt, 1987; Goodfellow *et al.*, 1992; Stackebrandt *et al.*, 1992). The branching hyphae of these saprophytic soil bacteria obtain nutrients and energy largely by degrading insoluble organic material through the production of a variety of extracellular hydrolytic enzymes (McCarthy & Williams, 1992). In response to appropriate signals, generally believed, but not proven, to include nutrient limitation, the substrate mycelium gives rise to aerial hyphae, which yield chains of uninucleoidal spores to provide a mechanism for dispersal and the colonization of new environments (Chater & Losick, 1996). Streptomyces also exhibit a remarkable capacity for biochemical differentiation, producing a wide variety of secondary metabolites that include about half of all known microbial antibiotics (Bérdy, 1984; Miyadoh, 1993). Many of these compounds have important applications in human medicine as antibacterial, antitumour and antifungal agents, and in agriculture as growth promoters, agents for plant protection (generally fungicides), antiparasitic agents and herbicides. Antibiotic production in streptomyces generally coincides with the onset of morphological differentiation, and the isolation of mutants that are deficient in both processes suggests at least some common elements of genetic control (Champness & Chater, 1994).

Within the last few years considerable progress has been made towards understanding the genetic and molecular basis of the regulation of antibiotic production by streptomyces. These studies have focused largely on *Streptomyces griseus*, the producer of streptomycin (reviewed by Horinouchi & Beppu, 1995, and Piepersberg, 1995), and on *Streptomyces coelicolor* A3(2) (reviewed by Chater & Hopwood, 1993; Hopwood *et al.*, 1995; Chater & Bibb, 1996). Analysis in *S. coelicolor* in particular has been assisted by the availability of natural and artificial genetic systems, and by the ability of the strain to produce

at least four antibiotics, enabling studies of pathway-specific and pleiotropic regulation of antibiotic production to be made. In addition, studies of the physiology and developmental biology (Hodgson, 1992; Chater, 1993; Champness & Chater, 1994; Chater & Losick, 1996) of *S. coelicolor* are yielding information that will be essential for a thorough understanding of the interactions that occur between primary and secondary metabolism, and for elucidating the regulatory networks that influence both morphological differentiation and antibiotic production.

In addition to its fundamental interest, a thorough knowledge of how the complex antibiotic biosynthetic pathways are regulated will inevitably allow for the development of more rational approaches to strain improvement (Chater, 1990). Although there is much to learn, this article summarizes work carried out in the author's laboratory that has contributed to our current understanding of the regulation of antibiotic production in *S. coelicolor*. Much of this information has arisen from the application of gene cloning to streptomyces. This same technology has revealed some unusual features of gene expression in streptomyces that will also be reviewed.

**Antibiotic production in streptomyces
occurs in a growth-phase-dependent manner**

Antibiotic production in streptomyces generally occurs in a growth-phase-dependent manner (Demain & Fang, 1995). For example, production of the anti-fungal antibiotic candicidin in liquid-grown cultures of *S. griseus* occurs after vegetative growth, estimated by monitoring DNA synthesis, has ceased [Fig. 1, from Martín & McDaniel, 1975; note that biomass continues to increase after the onset of candicidin production, presumably through the accumulation of storage compounds such as glycogen (Braña *et al.*, 1986) or triacylglycerol (Olukoshi & Packter, 1994)]. Similarly, in the perhaps more natural conditions of agar surface-grown cultures of *Streptomyces antibioticus*, oleandomycin production again occurs only after vegetative growth has finished (Fig. 2, from Méndez

Fig. 1. Production of candidicin by *S. griseus* in liquid culture. △, Candidicin concentration; ▲, dry weight cells; ●, glucose concentration; ○, DNA concentration. Redrawn from Martín & McDaniel (1975).

Fig. 2. Production of oleandomycin by agar surface-grown cultures of *S. antibioticus*. ●, Dry weight cells; ○, dry weight cells minus glycogen; △, oleandomycin concentration. Redrawn from Méndez *et al.* (1985).

et al., 1985); interestingly, synthesis of the antibiotic appears to be limited to the substrate mycelium, with production complete before the appearance of aerial hyphae.

S. coelicolor as a model streptomycete

A thorough understanding of how antibiotic production is regulated will require an integrated approach that combines genetics with biochemistry, physiology and molecular biology. The most genetically characterized and genetically manipulable streptomycete is *S. coelicolor* (Hopwood *et al.*, 1985a). This organism has a well-developed genetic and physical linkage map (Kieser *et al.*, 1992; Redenbach *et al.*, 1996), and, in contradiction to the paradigm of *Escherichia coli*, possesses a linear, not circular, chromosome of approximately 8 Mb (Lin *et al.*, 1993).

In addition, *S. coelicolor* possesses a number of accessory genetic elements, including at least two insertion sequences, IS110 and IS117 (Chater & Hopwood, 1993). It contains SCP1, a large linear plasmid of 350 kb (Kinashi & Shimaji-Murayama, 1991) that carries genes for production of the cyclopentanone antibiotic methylenomycin (Kirby & Hopwood, 1977) (still the only proven example of plasmid-determined antibiotic biosynthesis), and an integrative element, SLP1, that normally resides in a stable form in the chromosome, but which is capable of excision, conjugation and autonomous replication in other streptomycetes (Bibb *et al.*, 1981; Omer & Cohen, 1984; Brasch & Cohen, 1993). *S. coelicolor* also possesses a conventional circular plasmid, SCP2, which is self-transmissible and capable of mobilizing the chromosome (Bibb *et al.*, 1977; Bibb & Hopwood, 1981). High-frequency transfer of the plasmid into a lawn of recipient bacteria that lack the element causes inhibition of growth and sporulation of the recipient lawn. The resulting 'pocks', which were shown to be a general property of

self-transmissible streptomycete plasmids, provided the plasmid-determined phenotype that was used to develop the transformation system that is currently used worldwide (Bibb *et al.*, 1978). This technique is based on the addition of DNA, in the presence of PEG1000, to protoplasts made by treating mycelium with lysozyme in an osmotically stabilizing medium.

Some unusual features of gene expression in streptomycetes

The availability of a transformation system, and of readily isolable streptomycete plasmids and phages, led to the rapid development of DNA cloning systems (Bibb *et al.*, 1980; Suarez & Chater, 1980; Thompson *et al.*, 1980; reviewed by Hopwood *et al.*, 1987). The ability to clone streptomycete genes, and to examine their expression *in vivo*, revealed some of the basic features of gene expression in these organisms. It soon became apparent that streptomycetes possess a high degree of promoter sequence heterogeneity (Bibb *et al.*, 1985a; Janssen *et al.*, 1989; Janssen & Bibb, 1988, 1990; Strohl, 1992). Some of this variability reflects the occurrence of multiple σ factors, which confer on RNA polymerase core enzyme the ability to recognize different subsets of promoter sequences (Westpheling *et al.*, 1985; Buttner *et al.*, 1988). Thus far, at least eight different σ factors have been identified in *S. coelicolor* (Buttner, 1989; Chater *et al.*, 1989; Lonetto *et al.*, 1994; Potůčková *et al.*, 1995), and presumably more remain to be discovered.

Many streptomycete genes were found to be transcribed from more than one promoter (Bibb *et al.*, 1985b; Buttner *et al.*, 1987; Janssen & Bibb, 1988, 1990; Janssen *et al.*, 1989; Strohl, 1992), sometimes by more than one form of RNA polymerase holoenzyme (Buttner *et al.*, 1988; Westpheling & Brawner, 1989). Although the significance of this for the regulation of gene expression is not known, it may reflect changes in the availability of σ factors and other regulatory proteins during development. It also became apparent that a number of streptomycete mRNAs contravened the dogma of the time by lacking untranslated leader sequences (Janssen *et al.*, 1989; Bibb *et al.*, 1994; Strohl, 1992). Instead, they commenced either at, or one nucleotide before, the translation start codon, and therefore lacked conventional ribosome-binding sites, which show a degree of complementarity to a sequence close to the end of 16S rRNA (Stormo *et al.*, 1982). For example, the primary transcripts for the neomycin resistance gene (*aph*) of *Streptomyces fradiae* (Janssen *et al.*, 1989) and for the erythromycin-resistance gene (*ermE*) of *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) (Bibb *et al.*, 1994) start at the first nucleotide of the ATG start codon and one nucleotide before the GTG start codon, respectively. Presumably, sequence information internal to such mRNAs is responsible for ribosome recognition. Although the reason for this unusual situation is not clear, the majority of leaderless transcripts found so far in streptomycetes are derived from antibiotic-resistance genes that confer immunity to an antibiotic made by the strain, and it may be that these leaderless

mRNAs play an important role in the co-ordinate expression of antibiotic biosynthesis and resistance in the producing organism (Janssen, 1993).

The advent of DNA cloning in streptomycetes inevitably led to the rapid accumulation of nucleotide sequence data for a variety of genes, confirming the predictable consequences of the high G + C content of streptomycete DNA on codon usage (Wright & Bibb, 1992). With the G + C content of the third codon position averaging around 90 mol %, streptomycete genes possess a highly biased codon usage. Consequently, a number of codons ending with A or U occur very infrequently, and at least one of these, the leucine codon UUA, may have acquired a regulatory function (see below). Although the high G + C content of streptomycete DNA can make sequence determination difficult, it does offer one compensation – the highly biased codon usage results in a predictable and distinct distribution of G + C content across codons. This characteristic feature formed the basis of a simple and reliable computer program for the localization of protein coding sequences and for the identification of frameshift errors that is also applicable to other organisms with high G + C DNA (Bibb *et al.*, 1984).

Antibiotic biosynthetic genes occur in clusters that generally contain pathway-specific regulatory genes

It was not long before DNA cloning was applied to the study of antibiotic production. It was well-established that some streptomycetes made several different classes of antibiotics and that these compounds were generally the products of complex biosynthetic pathways. Although there was genetic evidence to indicate clustering of the biosynthetic genes, the ability to clone DNA in streptomycetes provided the first direct physical evidence for the extent of this clustering, and also served to identify the presence within these clusters of resistance determinants and pathway-specific regulatory genes.

This tight clustering was first demonstrated by cloning a 35 kb fragment of *S. coelicolor* DNA in *Streptomyces parvulus* that conferred production of the blue-pigmented polyketide antibiotic actinorhodin on its new host, which is not known to make any structurally related compounds (Malpartida & Hopwood, 1984). These studies were followed shortly afterwards by the first example of the use of DNA cloning to produce a new antibiotic – introduction of the *actV* genes from *S. coelicolor* into a *Streptomyces* strain that produced medermycin resulted in the synthesis of a novel hybrid compound, mederrhodin (Hopwood *et al.*, 1985b).

Gene cloning has since been used to study the regulation of antibiotic production, and it is this topic that is considered in the remainder of this article. Much of the following work, the aim of which is to decipher the physiological signals and underlying regulatory mechanisms that are responsible for the growth phase-dependence of antibiotic production in *S. coelicolor*, was

Fig. 3. Factors potentially determining the onset of antibiotic production in streptomycetes. Thinner lines represent plausible interactions for which there is currently no direct evidence.

conducted in the author's laboratory, and apologies are given to those whose work is not covered in similar detail (for additional information see Chater & Bruton, 1985; Adamidis *et al.*, 1990; Adamidis & Champness, 1992; Champness *et al.*, 1992; Fernández-Moreno *et al.*, 1992; Hobbs *et al.*, 1992; for reviews see Champness & Chater, 1994; Hopwood *et al.*, 1995; Chater & Bibb, 1996). Many factors appear to influence the onset of antibiotic production in streptomycetes (Fig. 3; Demain *et al.*, 1983; Demain, 1992; Demain & Fang, 1995; Chater & Bibb, 1996); some of these are considered below.

Pathway-specific regulatory genes play a pivotal role in determining the onset of antibiotic production in *S. coelicolor*

We have focused our attention on two of the four antibiotics made by *S. coelicolor* – the blue-pigmented polyketide actinorhodin (Act) and the chemically distinct red-pigmented tripyrrole undecylprodigiosin (Red). Under conditions of nitrogen limitation in liquid culture, Act and Red show classical production kinetics, with synthesis limited to stationary phase. By studying the regulation of both of these compounds, we can examine pathway-specific and pleiotropic regulation of antibiotic production. Many antibiotic-biosynthetic-gene clusters

contain pathway-specific regulatory genes, most of which appear to act as transcriptional activators (Chater & Bibb, 1996); for the Act and Red pathways, the genes are *actII-ORF4* (Fernández-Moreno *et al.*, 1991) and *redD* (Narva & Feitelson, 1990), respectively. Their products belong to an expanding family of regulatory proteins that includes the pathway-specific regulatory gene for daunorubicin production in *Streptomyces peucetius* (Stutzman-Engwall *et al.*, 1992), and the putative pleiotropic regulatory gene *afsR* (Horinouchi *et al.*, 1990). ActII-ORF4 and RedD show 33% amino acid sequence identity, but as one would expect, they are unable to substitute for one another even when over-expressed, although ActII-ORF4 and DnrI apparently can (Stutzman-Engwall *et al.*, 1992). In liquid culture, transcription of *redD* (Takano *et al.*, 1992) and of *actII-ORF4* (Gramajo *et al.*, 1993) occurs only during transition and stationary phase, and is followed shortly afterwards by transcription of the corresponding biosynthetic structural genes. If expression of either pathway-specific regulatory gene is forced during exponential growth, premature transcription of the corresponding biosynthetic structural genes results, and antibiotic production occurs while rapid growth continues. Thus, the principal limitation to antibiotic production, at least in these defined laboratory conditions, appears to be the availability of enough of the pathway-

specific activator protein. Over-expression of the pathway-specific regulatory genes increases markedly the amount of antibiotic made (Strauch *et al.*, 1991; Gramajo *et al.*, 1993).

Pleiotropic regulatory genes also control antibiotic production

In addition to pathway-specific regulatory genes, *S. coelicolor* possesses several genes with pleiotropic effects on antibiotic production. At least some of these are likely to play regulatory roles. The product of one, AfsR, is a homologue of ActII-ORF4 and RedD, with its N-terminal region showing 33 % amino acid sequence identity to each of the pathway-specific regulatory proteins. *afsR* was first identified through its ability to stimulate Act production in *Streptomyces lividans*, a close relative of *S. coelicolor* in which the *act* and *red* genes are normally poorly expressed (Horinouchi *et al.*, 1983). Recent work identified a small gene (*afsS*) located immediately downstream of *afsR* that encodes a protein of 63 amino acids which alone is capable of stimulating antibiotic production (Matsumoto *et al.*, 1995; Vögtli *et al.*, 1994). Re-examination of the earlier data does not contradict the hypothesis that *afsS*, and not *afsR*, is responsible for Act overproduction; moreover, the reduction in Act production that followed disruption of *afsR* could have reflected a polar effect on *afsS* expression. So is *afsR* really a regulatory gene for antibiotic production?

To address this question, high-copy-number plasmids that contained either *afsR* or *afsS* were made and both were shown to be capable of stimulating Act production in both *S. coelicolor* and *S. lividans*; however, the degree of stimulation mediated by *afsR* was significantly greater than that produced by *afsS* (Floriano & Bibb, 1996). Since the N-terminal region of AfsR is homologous to ActII-ORF4 and RedD, this might simply reflect regulatory cross-talk between AfsR and the pathway-specific activators when AfsR is expressed at high levels. However, the inability of *afsR* to suppress in-frame deletion mutations in *actII-ORF4* or *redD* even when cloned at high copy-number indicates that *afsR* cannot substitute for the pathway-specific regulatory genes. Consistent with this, a deletion in *afsR* that is in-frame and therefore unlikely to influence the expression of *afsS* results in the loss of Act and Red production on some media. Transcription of *actII-ORF4* and *redD* was not affected by the *afsR* mutation (Floriano & Bibb, 1996). So either AfsR influences Act and Red production independently of the pathway-specific regulatory proteins, or it is required for their activity, perhaps through their post-translational modification, or through the formation of a heteromultimeric protein complex. However it mediates its effects, *afsR* is clearly a true pleiotropic regulatory gene.

A second pleiotropic regulatory gene for antibiotic production in *S. coelicolor* is *afsB*. *afsB* mutants are defective in Act and Red production (Hara *et al.*, 1983), while synthesis of methylenomycin and the calcium-dependent antibiotic (CDA), the other two antibiotics known to be

made by the strain, appears to be unaffected (Adamidis & Champness, 1992). Efforts to complement *afsB* using a genomic library made in a low-copy-number plasmid led to the interesting discovery that additional copies of *hrdB*, which encodes the major σ factor of *S. coelicolor* (Brown *et al.*, 1992), restored Red and Act production in *afsB* mutants (Wietzorrek, 1996). The suppressive effect of *hrdB* resembles a recent report in *Pseudomonas fluorescens* (Schnider *et al.*, 1995), in which production of the antibiotics pyoluteorin and 2,4-diacetylphloroglucinol, which are made during stationary phase, was stimulated by the presence of additional copies of *rpoD*, which encodes the major and essential σ factor of that organism. Whether this reflects a role for the major σ factor of both organisms in the transcription of antibiotic biosynthetic genes, or results from an indirect effect (e.g. provision of precursors), remains to be determined (see below).

The third pleiotropic regulatory gene for antibiotic production that has been studied in our laboratory is *bldA* (see Champness & Chater, 1994, and references therein). On most laboratory media, *bldA* mutants have a very dramatic phenotype; not only are they deficient in aerial mycelium formation, giving rise to the term 'bald', but they are also pleiotropically deficient in antibiotic production, failing to produce any of the four *S. coelicolor* antibiotics. *bldA* encodes the only tRNA in *S. coelicolor* that can efficiently translate the rare leucine codon UUA (Lawlor *et al.*, 1987; Leskiw *et al.*, 1991a). The dependence of Act production on *bldA* was shown to be due to the presence of a single UUA codon in the *actII-ORF4* transcript; substitution of this codon on a plasmid-borne copy of *actII-ORF4* with the synonymous leucine codon UUG rendered Act production *bldA*-independent (Fernández-Moreno *et al.*, 1991). While control of transcription of *bldA* might seem to be a likely means by which to regulate growth-phase-dependent Act production, *bldA* transcripts are observed in minimal and rich liquid media throughout growth (Gramajo *et al.*, 1993). Furthermore, we were unable to demonstrate any limitation on the translation of UUA codons in rapidly growing liquid cultures (Gramajo *et al.*, 1993). Thus, while Act production is clearly *bldA*-dependent, we have been unable to demonstrate any regulatory role for *bldA*. In contrast, in studies conducted largely, but not entirely, with surface-grown cultures, Leskiw and colleagues *did* detect an increase in the level of *bldA* transcripts during growth, and limitations on the translation of UUA codons in young cultures (Leskiw *et al.*, 1991b, 1993); the differences in the two sets of results may reflect differences in the strains and media used. The precise role that *bldA* plays in regulating antibiotic production therefore remains to be determined.

In contrast to *actII-ORF4*, the transcript derived from *redD*, the pathway-specific activator gene for Red production, does not contain a UUA codon (Narva & Feitelson, 1990), so how do we explain the *bldA*-dependence of Red synthesis? The ability to relieve *bldA*-dependence of Act production simply by replacing the UUA codon of *actII-ORF4* with a synonymous UUG codon (Fernández-Moreno *et al.*, 1991) implied that

transcription of *actII-ORF4* was not *bldA*-dependent, and this has since been demonstrated experimentally in a series of S1 nuclease protection experiments using RNA isolated from otherwise isogenic *bldA*⁺ and *bldA* strains (J. White, personal communication). In contrast, transcription of *redD* was shown to be highly *bldA*-dependent (J. White, personal communication), a result that implied the existence of another gene that presumably contained a UUA codon and that was required for *redD* transcription. Recent studies (C. Flaxman, D. A. Hodgson & J. White, personal communication), which followed on from the isolation of second-site suppressor mutants of *bldA* in which Red production, but not Act synthesis or sporulation, was restored (E. Guthrie & K. F. Chater, personal communication), have identified a likely candidate for this additional pathway-specific regulatory gene, *redZ*, which encodes a homologue of the response regulator family of two-component regulatory systems (Msadek *et al.*, 1993), and which is located approximately 4 kb downstream of *redD*, contains a single UUA codon. Disruption of *redZ* results in loss of Red production and a marked reduction in the level of *redD* transcripts, suggesting that RedZ is a transcriptional activator of *redD* (J. White, personal communication). What regulates *redZ* expression? While the availability of a functional *bldA* tRNA remains a possibility, the results obtained with *actII-ORF4* suggest that other factors may play a determining role.

Small diffusible signalling molecules influence the onset of antibiotic production in *S. coelicolor*

Small diffusible signalling molecules play an important role in triggering antibiotic production in some streptomycetes (for reviews see Chater & Bibb, 1996, and Horinouchi & Beppu, 1992, 1995). For example, the γ -butyrolactone A-factor is required for streptomycin production and sporulation in *S. griseus* (Khokhlov *et al.*, 1967; Horinouchi & Beppu, 1995). *S. coelicolor* is capable of cross-feeding *S. griseus* mutants that are deficient in A-factor synthesis (Hara *et al.*, 1983), and makes a series of six related compounds (Anisova *et al.*, 1984; Efremenkova *et al.*, 1985), but not A-factor itself. In an attempt to identify a role for these compounds in antibiotic production, we have purified a compound detectable only in transition- and stationary-phase cultures of *S. coelicolor* that causes the precocious production of both Act and Red in the wild-type strain. The compound was purified using standard procedures for the isolation of γ -butyrolactones (E. Takano, personal communication), and it is likely that it is one of the six compounds identified by Efremenkova *et al.* (1985). It thus appears probable that γ -butyrolactones do indeed play a role in determining the onset of antibiotic production in *S. coelicolor*. Since the activity we detected was found only in transition- and stationary-phase cultures, it seems unlikely that these compounds act simply as quorum sensors, i.e. as indicators of cell density akin to the homoserine lactone derivatives made by *Vibrio* species (Fuqua *et al.*, 1994; Williams, 1994). Similar observations were made for the accumulation of virginiae butanolide C that stimulates virginamycin production in *Streptomyces virginiae* (Yang *et al.*

et al., 1995), and for A-factor production by *S. griseus* (Hara & Beppu, 1982). It thus appears more likely that the γ -butyrolactones are produced in response to certain physiological or environmental signals.

The highly phosphorylated nucleotide ppGpp may act as an intracellular effector for antibiotic production

A consistent feature throughout the *Streptomyces* literature is the likely role of a slowing or cessation of growth as a signal for determining the onset of antibiotic production. In *E. coli*, the highly phosphorylated nucleotide ppGpp appears to play a central role in the growth rate control of gene expression (Sarubbi *et al.*, 1988; Hernandez & Bremer, 1990, 1993; Schreiber *et al.*, 1991), and in the regulation of at least some genes that are expressed in stationary phase (Gentry *et al.*, 1993). Furthermore, *relC* mutants of a number of streptomycete strains that are defective in ppGpp synthesis are also impaired in antibiotic production, leading Ochi (1990) to suggest that ppGpp may play a key role in determining the onset of antibiotic biosynthesis. In our own studies, we generally see a positive correlation between ppGpp synthesis and transcription of *redD* (Takano *et al.*, 1992) and *actII-ORF4* (Strauch *et al.*, 1991) during transition and stationary phase of liquid-grown *S. coelicolor* cultures (Takano & Bibb, 1994). Moreover, if an exponentially growing culture is subjected to nutritional shiftdown by rapidly depleting it of amino acids, large amounts of ppGpp are produced, and transcription of *actII-ORF4* follows within minutes (Strauch *et al.*, 1991). Unfortunately, *relC* mutants deficient in ribosomal protein L11, which is apparently required for activation of the ribosome-bound ppGpp synthetase, are also impaired in growth and so it is difficult to know whether or not the reduction in antibiotic production reflects reduced levels of ppGpp or impaired protein synthesis. To assess more rigorously whether there might be a causal relationship between ppGpp synthesis and antibiotic production, we used a PCR-based approach to clone the ppGpp synthetase gene (*relA*) of *S. coelicolor* (Chakraborty *et al.*, 1996). The cloned gene was used to create a null-mutant that is totally deficient in ppGpp synthesis upon amino acid starvation (R. Chakraborty, personal communication). The resulting mutant grows at the same rate as the *relA*⁺ strain; it fails to make Act or Red on some media, but does so on others. We believe that this indicates a crucial role for ppGpp in antibiotic biosynthesis under some nutritional conditions.

The role of σ factor heterogeneity in antibiotic production in *S. coelicolor*

Although *S. coelicolor* contains at least eight σ factors (Buttner, 1989; Chater *et al.*, 1989; Lonetto *et al.*, 1994; Potůcková *et al.*, 1995), the functions of several are unknown. Given the role that alternative σ factors play in growth phase-dependent gene expression in other bacteria (Boylan *et al.*, 1993; Errington, 1993; Hengge-Aronis, 1993; Loewen & Hengge-Aronis, 1994), we set out to determine which form of RNA polymerase holoenzyme transcribes *redD* and *actII-ORF4*, and whether the avail-

ability of the associated σ factor might play a role in the regulation of antibiotic production. Analysis of RNA polymerase isolated from transition phase cultures of *S. coelicolor* indicated that both pathway-specific regulatory genes were transcribed by a holoenzyme that was different from the major form containing σ^{hrdB} (Fujii *et al.*, 1996; Brown *et al.*, 1992). Reconstitution experiments in which renatured components of the transition-phase holoenzyme preparations were added to *S. coelicolor* and *E. coli* core RNA polymerase identified a protein of apparent molecular mass 46 kDa that would elicit *in vitro* transcription from *redDp* and *actII-ORF4p* (Fujii *et al.*, 1996). Determination of the N-terminal amino acid sequence identified the protein as HrdD, a member of a family of four extremely similar proteins that includes the putative σ factors σ^{hrdA} and σ^{hrdC} , and the major and essential σ factor, σ^{hrdB} (Buttner *et al.*, 1990; Brown *et al.*, 1992; Tanaka *et al.*, 1991). However, disruption of *hrdD* had no effect on Red and Act production (Buttner *et al.*, 1990; Buttner & Lewis, 1992; Fujii *et al.*, 1996), indicating that *redD* and *actII-ORF4* are transcribed *in vivo* by at least one other form of RNA polymerase holoenzyme. Circumstantial evidence suggests that this is the major holoenzyme containing σ^{hrdB} . This apparent redundancy may perhaps reflect changing σ factor availability during growth and development.

Summary

The work described above summarizes our attempts to understand the genetic regulation of antibiotic production in *S. coelicolor*, and it should be apparent that there is much to learn. Nevertheless, we have characterized the expression of pathway-specific and pleiotropic regulatory genes, and we are beginning to understand the dependence relationships between them: but in no case do we know the molecular function of any of the gene products. We have isolated a diffusible factor, probably a γ -butyrolactone, that clearly influences the onset of antibiotic production, and we believe that we have identified a role for ppGpp as an intracellular effector for antibiotic production, consistent with an important role for growth rate in determining the onset of antibiotic production: but in neither case is the signal transduction pathway identified. Other factors that appear to influence the onset of antibiotic production, and that have not been considered here, include nutritional repression and inhibition (reviewed by Demain *et al.*, 1983; Demain, 1992; Demain & Fang, 1995), and imbalances in metabolism that may lead to physiological stress (Hood *et al.*, 1992; Hobbs *et al.*, 1992; G. Hobbs & S. Oliver, personal communication). All of the physiological parameters we have mentioned may act at different levels in a regulatory cascade or, as seems more likely, a complicated regulatory network, to effect the onset of antibiotic production. Such a complex regulatory web would be consistent with the need of the organism to sense, integrate and respond to a variety of environmental and physiological signals, and might explain the conditional nature of many of mutants that are pleiotropically defective in antibiotic production. In addition to *afsR*, *afsB* and *bldA*, there are other genes

that influence the production of two or more of the four antibiotics made by *S. coelicolor* (Adamidis *et al.*, 1990; Adamidis & Champness, 1992; Champness *et al.*, 1992; Fernández-Moreno *et al.*, 1992; Hong *et al.*, 1991; Horinouchi, 1993; Ishizuka *et al.*, 1992; for reviews see Champness & Chater, 1994; Hopwood *et al.*, 1995; Chater & Bibb, 1996). Some of these (the *bld* genes) are also required for morphological differentiation. Understanding the physiological and environmental conditions that trigger the expression of these genes, or that influence the activities of their products, and the corresponding signal-transduction pathways that are responsible for the activation of antibiotic biosynthesis remains a major goal for the future.

Acknowledgements

I would like to thank past and present members of my laboratory who have carried out much of the work I have described, and in particular Rekha Chakraborty, Belén Floriano, Takeshi Fujii, Hugo Gramajo, Eriko Takano, Janet White and Andreas Wietzorrek for allowing me to quote their unpublished data. I also thank Christine Flaxman, David Hodgson, Ellen Guthrie, Keith Chater, Glyn Hobbs and Steve Oliver for permission to describe their unpublished work, and Mark Buttner, Keith Chater and David Hopwood for their comments on the manuscript. Finally, I would like to thank colleagues in the *Streptomyces* group at the John Innes Centre for their continuing interest and support.

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