Chapter 5
Streptomyces Bacteria: Specialized Metabolism, Inter-species Interations and Non-coding RNAs

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Abstract *Streptomyces* bacteria are abundant in soil environments, where they have an unusual multicellular life cycle that involves filamentous growth and spore formation. They also produce an extraordinary range of compounds known as specialized metabolites, and it is through these compounds that they interact with many of their terrestrial neighbours. Specialized metabolite production is subject to a wide range of regulatory inputs, and it is predicted that non-coding RNAs are amongst the many regulators governing metabolic output. RNA-sequencing experiments have revealed many non-coding RNAs expressed within specialized metabolic clusters of diverse *Streptomyces* species, with antisense RNAs featuring prominently. Here, we highlight a number of specialized metabolites whose gene clusters contain known non-coding RNAs, and consider possible roles for these RNA regulators in influencing *Streptomyces* interactions with other organisms in the environment.

5.1 Introduction

The soil is home to a diverse community of organisms that encompasses everything from the microscopic (e.g. bacteria and fungi) to the macroscopic (e.g. plants and insects). Among these organisms, the soil-dwelling actinobacteria, notably the streptomycetes, are the most ubiquitous of the soil bacteria. Streptomycetes are best known for their ability to produce a multitude of medically useful compounds, and for their complex developmental cycle. Unlike most bacteria, *Streptomyces* have a multicellular life cycle, which involves a series of defined, differentiated stages (Fig. 5.1). *Streptomyces* are sporulating bacteria, and following spore germination,

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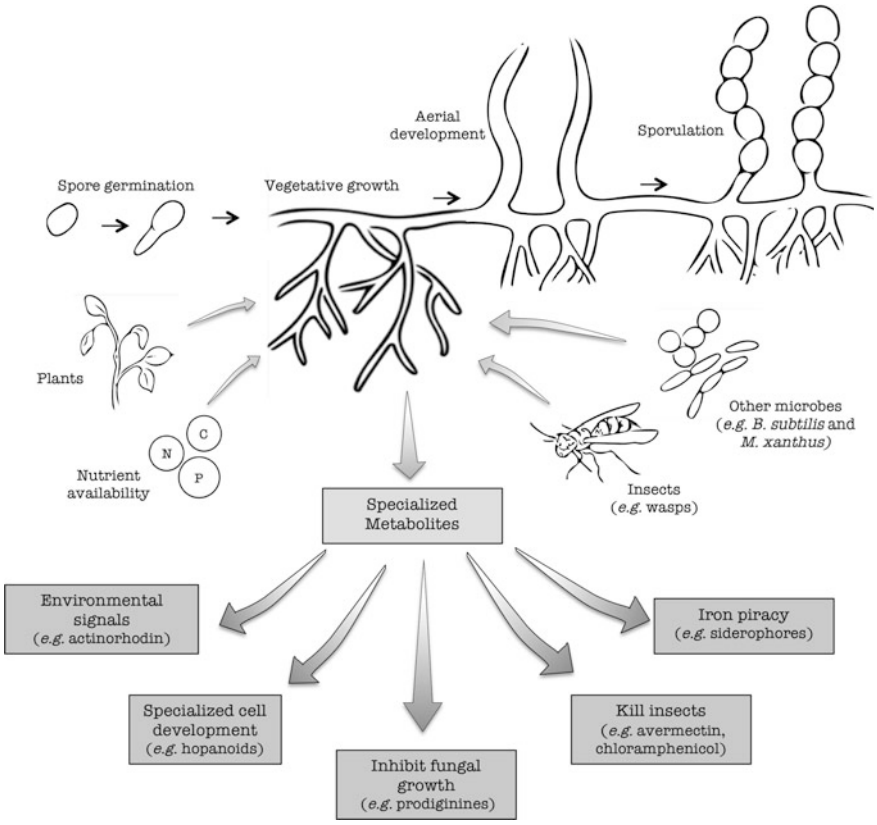


Fig. 5.1 ***Streptomyces*** **life cycle, and interactions that affect specialized metabolite production.** When *Streptomyces* spores encounter favourable growth conditions, they germinate. Germ tubes grow by hyphal tip extension and branching, generating a dense vegetative mycelial network. In response to environmental cues (such as those indicated below the life cycle), aerial hyphae extend upwards and eventually septate into spores. These spores can then be dispersed, and the cycle can begin anew. Specialized metabolite production typically initiates during the transition from vegetative growth to aerial development, and these can both mediate inter-species (and inter-kingdom) interactions, and confer growth benefits to *Streptomyces* by helping to obtain new nutrients or promote developmental progression

they grow via hyphal tip extension and branching to form a filamentous network of cells known as the vegetative mycelium. When nutrients become limiting, or in response to as yet unknown environmental cues, reproductive growth is initiated, with unbranched filaments first extending from the vegetative cells, rising into the air. These aerial filaments then undergo a synchronous round of septation to form chains of unigenomic spores (Flärdh and Buttner 2009; McCormick and Flärdh 2012).

During the *Streptomyces* life cycle, the transition from vegetative to aerial growth coincides with the onset of specialized (or secondary) metabolite production (Fig. 5.1). These metabolites differ from primary metabolites in that they are not

required for *Streptomyces* vegetative growth. Instead, their production is expected to confer a fitness benefit in their natural environment, and based on the coordination of their regulation with aerial hyphae formation, they are expected to be most important during the reproductive (sporulation) phase of growth. Virtually all *Streptomyces* species examined to date have the genetic ability to produce at least 20–30 distinct specialized metabolites (Traxler et al. 2013). These metabolites have been broadly co-opted for use in medicine and agriculture: many are employed as antibiotics, whilst others have utility as anti-fungals, anti-parasitic compounds and chemotherapeutic agents (Nett et al. 2009). Despite the importance of these compounds to clinical and veterinary medicine, we know little about their ecological roles.

Interactions between different organisms are inevitable in the diverse soil environment, and these can take the form of cooperative or competitive associations. For *Streptomyces* bacteria, specialized metabolites are predicted to mediate interactions with other organisms in the environment (Fig. 5.1). It has long been thought that these metabolites are agents of inter-species competition, whereby *Streptomyces* release chemical weapons to defend their territory in response to nutrient limitation. Recent findings—driven by the realization that antibiotics rarely reach inhibitory levels in the soil, yet can still induce differential transcription in nearby species—have prompted speculation that these specialized metabolites may serve as signaling molecules (Davies 2013). Whether *Streptomyces* specialized metabolites act as weapons or signals, they play a central role in mediating a myriad of interactions in the heterogeneous soil environment. Recent work has revealed that associations with other bacteria can alter the secreted metabolome of *Streptomyces* (Abrudan et al. 2015; Traxler et al. 2013), supporting the notion that these specialized metabolites mediate interactions with other bacteria, and likely other soil organisms including fungi, insects and plants. Although several remarkable examples of streptomycete-eukaryotic interactions have been described (Barke et al. 2010; Bignell et al. 2010; Currie et al. 1999; Kaltenpoth et al. 2005), much remains to be elucidated about the dynamics and consequences of these inter-kingdom interactions. Here, we consider how *Streptomyces* non-coding RNAs—key regulatory elements that play multifaceted roles in bacteria—could affect the activity/secretion of specialized metabolites, and how their effects may influence *Streptomyces* interactions with organisms ranging from other bacteria to higher order eukaryotes.

5.2 Regulation of Specialized Metabolite Production

The production of any given specialized metabolite is driven by gene products encoded from a discrete gene cluster in the *Streptomyces* chromosome (Fig. 5.2). These metabolic gene clusters direct the expression of the enzymes needed to synthesize the core metabolite, along with those that modify and tailor the

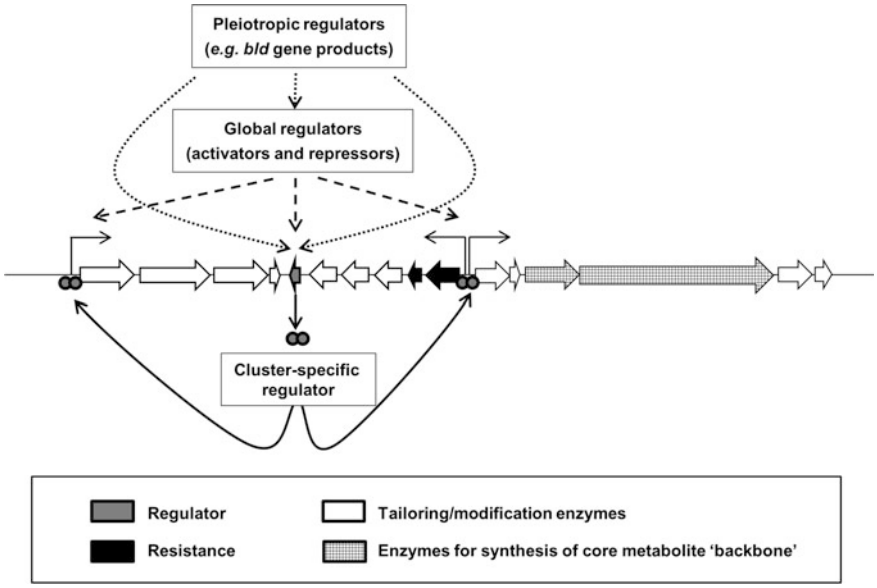
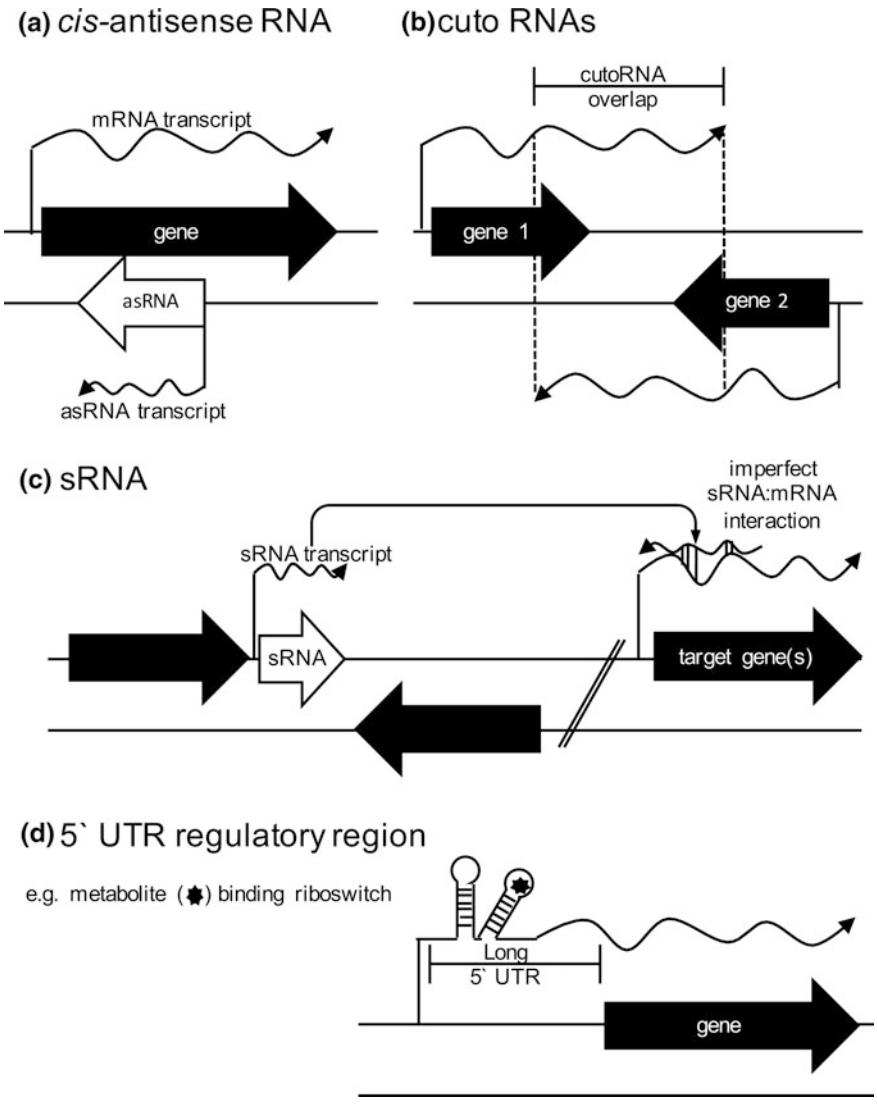


Fig. 5.2 Hierarchy of protein-based regulation of gene expression within a specialized metabolite biosynthetic cluster. Specialized metabolite biosynthetic clusters typically contain all the genes whose products are needed to synthesize the metabolite ('core' and 'tailoring' enzymes), as well as those encoding regulators and resistance/efflux determinants. These clusters are subject to extensive regulation. The most basic level of control is exerted by the cluster-specific regulators, which are encoded within the biosynthetic cluster whose expression they control. Global regulators are expressed from locations outside these biosynthetic clusters, and often affect the expression of more than one cluster; they can directly influence the expression of the biosynthetic genes, or, more commonly, the cluster-specific regulator. Pleiotropic regulators control both development and specialized metabolite production, and the best characterized of these are the *bld* gene products (Flärdh and Buttner 2009)

molecule. In addition, these clusters also direct the expression of regulatory proteins and resistance/secretion determinants (e.g. efflux pumps). Perhaps not surprisingly, specialized metabolite production is a highly regulated process, integrating diverse regulatory inputs including a wide range of nutritional cues (e.g. carbon, nitrogen and phosphorus availability) and the proximity of other organisms (Fig. 5.1). Specialized metabolic gene clusters are subject to control by different mechanisms, including pleiotropic regulators that couple metabolite production with aerial development, global metabolic regulators that influence the production of multiple metabolites, and cluster-specific regulators that activate/repress the expression of individual metabolites (Fig. 5.2) (reviewed in Bibb 2005). More recently, there have been reports suggesting that non-coding RNAs (ncRNAs) may also play key roles in governing the production of specialized metabolites (Moody et al. 2013).

5.3 Regulation by Non-coding RNAs

The regulatory roles ascribed to ncRNAs have grown tremendously over the last decade, and many classes of ncRNAs have now been identified in bacteria. These include antisense RNAs (asRNAs), *trans*-encoded small RNAs (sRNAs) and riboswitches (Fig. 5.3). All but the sRNAs have been associated with specialized



◀ **Fig. 5.3 Schematic diagram of the different classes of bacterial non-coding RNAs**. Protein-coding genes are depicted as *black arrows* and non-coding RNAs are shown as *white arrows*. RNA transcripts are shown above their corresponding gene, with transcription initiating at the *vertical line*, and terminating at the *small arrowhead*. **a** *cis*-antisense RNAs (referred to here as simply antisense RNAs or asRNAs) are transcribed on the strand, opposite a protein-coding gene. **b** cutoRNAs are a subset of asRNA, and arise when the 3′ UTR of an mRNA overlaps with that of a convergently-oriented downstream gene. The region of overlap is indicated with a bracketed line. **c** Intergenic *trans*-encoded small RNA (sRNA) genes are found in the intergenic regions between genes, and typically target (by imperfect complementary base-pairing) one or more mRNAs expressed from disparate chromosomal locations. **d** Long 5′ UTRs can be subject to a wide range of regulatory effects, with riboswitches being amongst the best-studied. Riboswitches adopt structures that permit the specific recognition and binding of small metabolites, leading to a change in the transcriptional read-through or translation of the associated mRNA. Other long 5′ UTRs can be targeted by sRNAs or RNA binding proteins, which alter the stability or translatability of the associated mRNA

metabolic clusters, and the current lack of sRNA-mediated regulation is almost certain to change as our understanding of these regulators grows.

In the case of asRNAs, these are most commonly expressed from the strand opposite a protein-coding gene, and consequently, they share perfect complementarity with their corresponding mRNA target (Georg and Hess 2011) (Fig. 5.3). In binding to their target mRNAs, they can affect mRNA stability (positively or negatively), and/or influence translation by modulating ribosome accessibility. Similar regulatory outcomes (transcript stability and mRNA translatability) have been ascribed to the sRNAs, which are so named for their relatively small sizes (~50–300 nt). asRNAs can also arise from the transcriptional read-through of convergently oriented genes that lack transcriptional terminators, such that there is considerable overlap of their 3′ untranslated region (and in some instances, overlap extending into the coding sequence) (Fig. 5.3, and below). We have termed this subset of asRNAs 'cutoRNAs' (for convergent untranslated overlapping RNAs) (Moody et al. 2013). While it is possible to envision a multitude of regulatory outcomes stemming from such overlap (e.g. coordinated degradation; coordinated translation), there is not yet any experimental evidence supporting such a role for these ncRNAs.

Unlike asRNAs, sRNAs are expressed from intergenic regions, and act in *trans* to target one or more mRNAs through imperfect base-pairing (Fig. 5.3). Often the secondary structures of sRNAs and mRNAs play an important role in facilitating the interaction. In many bacterial species, productive sRNA-mRNA interactions require the activity of the RNA chaperone Hfq; however, there is no such chaperone found in the streptomycetes (Jousselin et al. 2009). A handful of sRNAs have been shown to target proteins in place of mRNAs, where they act as molecular decoys, sequestering proteins from their native targets, thus inhibiting their activity (Waters and Storz 2009).

asRNAs and sRNAs are typically expressed as discrete genetic entities. In contrast, riboswitches are structured RNA elements usually found at the 5′ end of an mRNA. They adopt different conformations in the presence/absence of a ligand,

and serve to modify translation, transcription or stability of the downstream *cis*-encoded mRNA (Fig. 5.3) (reviewed in Montange and Batey 2008).

To date, hundreds of ncRNAs have been identified in model *Streptomyces* species, and they appear to be abundant in specialized metabolic gene clusters (Moody et al. 2013; Swiercz et al. 2008; Vockenhuber et al. 2011). Given the potential for specialized metabolites to serve as communication signals and/or chemical weapons, and given the role of ncRNAs in rapidly altering genetic programs in response to environmental changes, it is easy to imagine situations whereby ncRNAs would modulate inter-species and inter-kingdom interactions. Below, we highlight a number of specialized metabolites whose gene clusters contain known ncRNAs, and consider possible roles for these RNA regulators in affecting *Streptomyces* associations with other organisms in the environment.

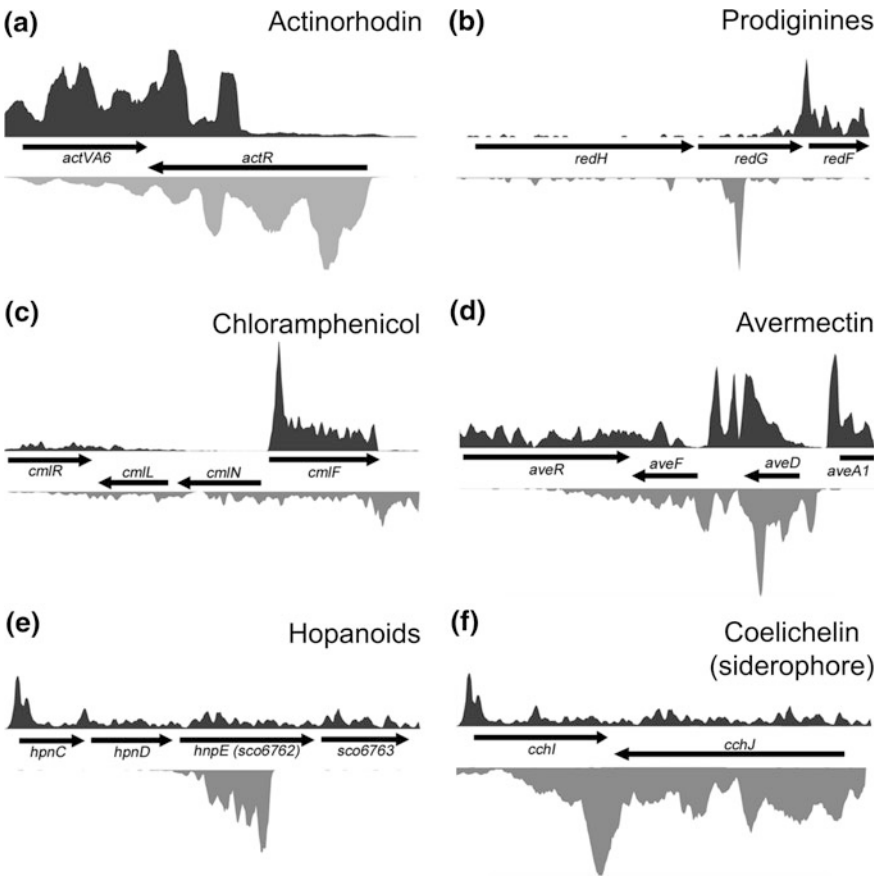
5.4 Actinorhodin—A Redox-Active Pigmented Antibiotic Produced by *Streptomyces coelicolor*

The best studied streptomycete, *Streptomyces coelicolor*, is so named for its characteristic blue colouration (coelicolor = sky blue). This is due to the production of a blue-pigmented specialized metabolite known as actinorhodin. In the environment, actinorhodin is secreted from the cell, where it can either diffuse freely, or be packaged into extracellular vesicles (Schrempf et al. 2011). It has antibiotic activity, specifically inhibiting the growth of nearby Gram-positive bacteria, and can also serve as a signalling molecule, where it is recognized and bound by receptors in other bacteria, altering the transcription profiles of these organisms (Xu et al. 2010). Actinorhodin production is further stimulated by the nearby growth of other microbes, including *Myxococcus xanthus*, *Bacillus subtilis*, and *Serratia* species. *M. xanthus* is a predatory soil microbe, and would inhabit the same environmental niche as *Streptomyces,* but as a Gram-negative bacterium, it would be impervious to the antibiotic activity of actinorhodin. Intriguingly, actinorhodin curtails *M. xanthus* predation of *S. coelicolor*, suggesting that in addition to its antibiotic and signalling properties, actinorhodin may also modulate the behaviour of other microbes through its action as a repellent (Perez et al. 2011).

The regulatory networks governing actinorhodin biosynthesis are the best understood of any specialized metabolic cluster studied to date. Expression of its cluster-specific regulator (ActII-ORF4) is directly controlled by multiple pleiotropic and global regulators (Fernandez-Moreno et al. 1991; Gao et al. 2012; McKenzie and Nodwell 2007; Rigali et al. 2008; Uguru et al. 2005; Wang et al. 2013) (e.g. Fig. 5.2). A second regulator within the actinorhodin biosynthetic cluster, ActR, directs the expression of several transporter genes, whose products are thought to confer self-resistance to actinorhodin by exporting it from the cytoplasm. Remarkably, ActR coordinates both actinorhodin synthesis and efflux: as a TetR-like regulator, ActR represses the expression of its divergently expressed

target genes (Tahlan et al. 2007) until it binds either actinorhodin or an actinorhodin precursor. Ligand binding results in a conformational change in ActR that inhibits its DNA binding ability, ultimately leading to efflux gene expression (Xu et al. 2012).

While there are currently no *trans*-encoded sRNAs known to affect actinorhodin production, nor any independently-encoded asRNAs identified within the biosynthetic cluster, there is a striking cutoRNA shared by the *actR* gene and the convergently expressed *actVA-5/6* operon (Fig. 5.4a). The *actR* and *actVA-6* genes share 34 nucleotides of sequence overlap at their 3′ ends, and transcription from *actR* and the *actVA-6*-containing operon extends the full length of their respective downstream genes (Fig. 5.4a). A similar organization is found in other specialized metabolic clusters [e.g. the simocyclinone (DNA gyrase-targeting antibiotic) biosynthetic cluster in *Streptomyces antibioticus* (Le et al. 2009)]. It is tantalizing to speculate that this genetic overlap, and corresponding transcriptional overlap, provides a means of physically coupling the final steps in antibiotic production



◀ **Fig. 5.4 RNA-seq expression profiles of select non-coding RNAs associated with specialized metabolite biosynthetic clusters**. Genes are shown schematically as *black arrows*. Above these, the *dark grey* coloured graphs represent relative read coverage for the forward strand, while the *bottom*, *light grey* graphs represent read coverage for the reverse strand. **a** Within the actinorhodin biosynthetic gene cluster in *S. coelicolor*, the lack of transcriptional termination for both the *actR* gene (encoding an efflux pump regulator) and the convergently expressed *actVA-6* operon (encoding tailoring enzymes), results in the formation of a cutoRNA. **b** An asRNA is expressed opposite *redG* (encoding a Rieske oxygenase-like enzyme) in the prodiginine biosynthetic gene cluster of *S. coelicolor*. **c** The chloramphenicol biosynthetic cluster of *S. venezuelae* contains many non-coding RNAs. There is an asRNA expressed opposite *cmlF* (efflux pump), and cutoRNAs are expressed from the convergently arranged *cmlR* (cluster-specific activator) and the *cmlLN* operon (likely biosynthetic enzyme and efflux pump, respectively). **d** Many non-coding RNAs are located within the avermectin biosynthetic gene cluster of *S. avermitilis*. cutoRNAs arise from the convergently transcribed *aveR* (cluster-specific regulator) and *aveF* (tailoring enzyme). Numerous asRNAs, are expressed opposite the *aveDF* operon, where both these genes specify tailoring enzymes. And the highly expressed 5′ UTR of *aveA1*, which encodes a polyketide synthase (core biosynthetic enzyme), may have a regulatory function, given the differential expression of the UTR relative to the coding sequence. **e** Within the hopanoid biosynthetic gene cluster of *S. coelicolor* an asRNA is expressed opposite *sco6762* (product of unknown function). **f** A cutoRNA is created by the convergent transcription of two genes (*cchJ*/*cchI*) encoding a transporter/biosynthetic enzyme, respectively, in the coelichelin siderophore biosynthetic gene cluster

with antibiotic export. With actinorhodin, this would directly connect the ActVA-6-mediated production of an actinorhodin precursor and dimerization of this intermediate molecule by ActVA-5 (Sciara et al. 2003; Valton et al. 2008), with the regulator that senses both precursor molecules and the final actinorhodin product. The complementarity shared by these mRNAs may ensure that the resulting translated products are coordinately synthesized in space and time, or may alter the stability and/or translation of either transcript. The cutoRNA connection with actinorhodin export would link it directly to the secretion of this antibiotic into the environment, where it would be available to exert its effects on any nearby organisms.

5.5 Prodiginines—Fungal Activity Modulators

In addition to actinorhodin, *S. coelicolor* also produces a family of red-pigmented specialized metabolites known collectively as the prodiginines. These compounds have antibacterial, antifungal, antimalarial, immunosuppressive and anticancer activities. *S. coelicolor* produces a mixture of prodiginines, including undecyl-prodigiosin (Red) and its cyclic derivative butyl-meta-cycloheptylprodiginine (streptorubin B) (Williamson et al. 2006). The wide range of bioactive properties ascribed to the prodiginines has prompted considerable speculation about the ecological function of these molecules. Similar to actinorhodin, prodiginine

production by *S. coelicolor* is stimulated upon interactions with other bacteria, including association with live *Bacillus subtilis* (Traxler et al. 2013; Yang et al. 2009), heat-killed *B. subtilis* or *Staphylococcus aureus* (Luti and Mavituna 2011), and live mycolic acid-containing bacteria (Onaka et al. 2011). A recent study revealed antibiotics produced by other streptomycetes (specifically, jadomycin B produced by *Streptomyces venezuelae*) can induce *S. coelicolor* prodiginine production (Wang et al. 2014). In each of these cases, it is not known how or why prodiginine production is induced. It has been proposed that prodiginines are produced in response to competition for nutrients with other bacteria; it is conceivable that fungi sharing streptomycete territory could similarly induce prodiginine synthesis. The induction of prodiginine production by jadomycin B further suggests other antibiotics—including those produced by fungi—could stimulate prodiginine release (Traxler and Kolter 2015).

Meschke et al. (2012) recently demonstrated the first direct role for prodiginines in an inter-kingdom interaction. The authors showed prodiginines produced by *Streptomyces lividans* could suppress the growth of the fungus *Verticillium dahlia*, the causative agent of vascular wilt in over 200 plant species including *Arabidopsis thaliana*. Co-cultivation of *S. coelicolor* and *V. dahlia* led to enhanced prodiginine expression, and a corresponding reduction in *V. dahlia* infection of *A. thaliana* (Meschke et al. 2012). While the benefit of prodiginine production for *S. lividans* in this case is unclear, the co-existence of microbes and plants in the rhizosphere suggests that a complex network of prodiginine-fungal-plant interactions could influence the growth and behaviour of multiple species.

Prodiginine production in *S. coelicolor* is directed by the *red* biosynthetic cluster, and yields a 2:1 mixture of undecylprodigiosin:streptorubin B (Williamson et al. 2006). The *red* cluster consists of 22 genes, organized into four transcriptional units, with the *redG* product, a Rieske oxygenase-like enzyme, catalyzing the conversion of undecylprodigiosin to streptorubin B. An asRNA is encoded on the strand opposite *redG* (Fig. 5.4b), and this ncRNA could conceivably provide a means of modulating the undecylprodigiosin:streptorubin B ratio, without affecting the expression of downstream genes whose products are required at earlier stages in the undecylprodigiosin biosynthetic pathway (Moody et al. 2013). *S. lividans* has a near identical cluster to that of *S. coelicolor,* raising the possibility that an equivalent asRNA may control undecylprodigiosin:streptorubin B levels in this species as well. In *S. lividans,* as in *S. coelicolor,* undecylprodigiosin levels are typically higher than those of streptorubin B, and levels of both molecules were increased by $\sim$4× during co-cultivation with *V. dahlia* (Meschke et al. 2012). It would be interesting to determine whether interactions with different fungi (or bacteria) could alter the levels of the *redG* asRNA, thus skewing the undecylprodigiosin:streptorubin B ratio.

5.6 Chloramphenicol

As mentioned briefly above, *S. venezuelae* can stimulate prodiginine production through its secretion of the antibiotic jadomycin B. *S. venezuelae* is, however, better known for its production of chloramphenicol, which was the first antibiotic to be synthesized on a large scale for clinical use. Although the therapeutic use of chloramphenicol has declined due to its adverse side-effects, its broad spectrum activity (against both Gram-positive and Gram-negative bacteria) means it is still prescribed in some circumstances (Nitzan et al. 2010). A recent study revealed that *S. venezuelae* produces appreciable amounts of chloramphenicol in its natural soil environment (Berendsen et al. 2013). Despite its high natural occurrence in soils, the role played by chloramphenicol in inter-bacterial and inter-kingdom interactions has not been directly explored.

Chloramphenicol inhibits protein synthesis by binding the 50S ribosomal subunit in bacteria and mitochondria. Blocks in mitochondrial protein synthesis, such as those associated with antibiotic inhibition of the 50S subunit, can result in decreased ATP biosynthesis and generalized mitochondrial stress, and this in turn can be associated with accelerated human cancer progression (Li et al. 2010). Given the relative abundance of chloramphenicol in the soil, it seems likely that *S. venezuelae*, and other producing bacteria, could use chloramphenicol as a chemical weapon to induce mitochondrial stress in eukaryotes. This could provide a fitness benefit to *S. venezuelae* during competition with fungi for nutrients, and indeed, supporting this proposal is the observation that chloramphenicol can inhibit the growth of the soil fungus *Spocullariopsis* (now *Microascus*) *brevicaulis* (Broadbent and Terry 1958).

The chloramphenicol biosynthetic cluster in *S. venezuelae* comprises 15 protein-coding genes and includes several prominent asRNAs (Fig. 5.4c). One of these is expressed opposite the gene encoding the chloramphenicol efflux pump CmlF. It is thought that CmlF directs the secretion of chloramphenicol into the environment, and modulating levels of its asRNA might provide a way of altering chloramphenicol release in response to the presence of bacteria or fungi (Moody et al. 2013). The other asRNAs are cutoRNAs expressed from a region containing three protein-coding genes that were recently shown to be part of the chloramphenicol cluster: *cmlR*, a transcriptional activator of the cluster; *cmlL*, a phosphopantetheinyl transferase; and *cmlN*, a Na^+/H^+ antiporter (Fernandez-Martinez et al. 2014). The transcriptional organization in this region is strikingly complex, and is analogous to that of *actR* and the *actVA-6* operon in *S. coelicolor*. *cmlL* and *cmlN* are transcribed as an operon, and this operon is convergently oriented relative to *cmlR*. The 3′ UTR of the *cmlLN* transcript extends throughout the entire *cmlR* encoded region, and likewise, the 3′ UTR of the *cmlR* transcript extends throughout the majority of the *cmlLN* operon. Given that CmlR activates the cluster and is required for chloramphenicol production, while CmlN is thought to be involved in chloramphenicol export, both asRNAs might be able to rapidly modulate chloramphenicol production and export in response to changes in environmental conditions (Moody et al. 2013).

5.7 The Avermectin Cluster in *Streptomyces avermitilis*

The anti-eukaryotic properties of chloramphenicol are not unique to this metabolite. *Streptomyces avermitilis* produces a group of macrocyclic lactones known as avermectins, which are highly toxic to insects and nematodes. These toxic properties have led to the pervasive use of avermectins as anti-parasitic agents in human and veterinary medicine (Omura and Crump 2004). Avermectins act to paralyze—and ultimately kill—insects, by disrupting glutamate-gated chloride channels that are critical for neurotransmission (Arena et al. 1991; Cully et al. 1996; Kane et al. 2000). Why *S. avermitilis* produces avermectins in its natural environment is not known, but *Streptomyces*-insect associations have been well documented. These interactions are, however, more typically in the form of symbiotic relationships with e.g. wasps and termites, where the insect provides *Streptomyces* with nutrients, and *Streptomyces* in turn, supplies an arsenal of specialized metabolic weapons to ward off microbial pathogens (Seipke et al. 2012). It is conceivable that similar relationships have evolved in which *S. avermitilis* produces avermectins to protect a host from enemy insects.

In *S. avermitilis,* the avermectin biosynthetic gene cluster spans ~81 kb and includes 19 protein-coding genes. While ncRNAs abound within this cluster, there is an 18 kb region in which they are particularly enriched (Fig. 5.4d). This region contains genes that contribute to the regulation, synthesis and tailoring of the avermectins (Ikeda et al. 2003; Omura et al. 2001), and includes genes coding for the cluster-specific activator AveR, two polyketide tailoring enzymes (AveF and AveD), and the first of four polyketide synthases (AveA1) [polyketides are a class of specialized metabolites that are produced by enzyme complexes having similarity to fatty acid synthases]. In addition to these protein-coding genes, this region encompasses multiple asRNAs, a significant cutoRNA, and a long 5′ UTR that may have regulatory potential (Fig. 5.4d).

aveR and *aveF* are convergently transcribed but lack intergenic transcriptional terminators. This organization provides an opportunity to generate a long cutoRNA, as a result of read-through transcription for each gene. In the absence of temporal data, it is not yet clear whether these genes are expressed at the same time, and if they are, what benefit may arise from their transcriptional overlap. Upstream of *aveF* and its co-transcribed *aveD* gene, there exist several highly expressed asRNAs, which may function to alter the relative amounts of AveD and AveF accumulating in the cell. Finally, the 5′ UTR of *aveA1* is unusually long (130 nt), suggesting that there may be regulatory elements (e.g. riboswitch or sRNA/protein regulator binding sites) contained within this region (Moody et al. 2013). It has yet to be determined how these ncRNAs impact avermectin production; however, the transcriptional complexity in this small region suggest that ncRNA-mediated regulation likely has profound effects on avermectin production.

5.8 Antisense RNA Control of Hopanoid Production—A Role in Altering Membrane Properties

While many specialized metabolites are produced in a largely species-specific manner, including actinorhodin and avermectin, there are others that are produced by many bacterial species, like the hopanoids and siderophores (see below). Hopanoids are pentacyclic compounds produced by bacteria, and are considered to be functionally equivalent to eukaryotic sterols (Saenz et al. 2012). Like sterols, hopanoids alter membrane function, specifically affecting membrane fluidity and permeability (Wu et al. 2015). In other environmental bacteria (specifically *Burkholderia*), hopanoid production enhances resistance to antibiotics and detergents, and promotes survival in the face of acidic pH, temperature fluctuations and changes to osmotic pressure (Malott et al. 2012; Schmerk et al. 2011). In *Streptomyces*, hopanoids do not seem to promote resistance to exogenous stresses (Seipke et al. 2012), although the hopanoid gene cluster in *S. coelicolor* is under the control of the thiol/oxidative stress-responsive sigma factor SigR, suggesting a role in mitigating oxidative or redox stress (Kim et al. 2012). The primary function of hopanoids in *Streptomyces,* however, seems to be during sporulation, where they are proposed to minimize water loss, and thus promote spore viability (Poralla et al. 2000). This is consistent with the observation that these molecules are produced predominantly during sporulation on solid media (Moody et al. 2013; Poralla et al. 2000). This role in helping promote the formation of specialized cell types (like spores) seems to be conserved throughout the actinobacteria. In the streptomycetes, spore formation permits survival during adverse growth conditions, and this dormant state offers immunity to many of the chemical insults levied against them by other bacteria and fungi.

The hopanoid biosynthetic cluster in *S. coelicolor* is predicted to encompass 12 genes (Bentley et al. 2002; Liu et al. 2014; Pan et al. 2015; Seipke et al. 2012) (Fig. 5.4e). In examining the transcriptional profile of the hopanoid cluster in several streptomycetes, a strong asRNA was observed to be expressed opposite *sco6762* in *S. coelicolor* and its orthologues in other species. In *Frankia*, an actinobacterium and nitrogen-fixing plant symbiont, hopanoids contribute to the development of nitrogen-fixing nodules within plant roots, facilitating the formation of an oxygen-impermeable barrier around the nitrogenase enzyme (Berry et al. 1993). This gene (*sco6762*) is conserved in *Frankia*, and perhaps with it, the asRNA. The product of SCO6762 is most similar to that of HpnE, an enzyme predicted to convert hydroxysqualene to squalene (Pan et al. 2015), where squalene is an intermediate in hopanoid biosynthesis. Based on what is known about asRNA activity in other systems, we predict this asRNA may function to negatively regulate the levels of SCO6762, and possibly decouple the expression of this gene (and those downstream in the operon) from the hydroxysqualene biosynthetic genes upstream. This may allow the cells to direct the metabolites produced by the upstream HpnC/HpnD gene products into other pathways, thereby downregulating hopanoid production. It will be interesting to determine whether expression of the

asRNA is limited to cells in which hopanoid production is less important, such as in *Frankia* cells not associated with the nodule/nitrogenase production, and in *Streptomyces* vegetative (non-sporulating) cells.

5.9 Siderophores—Production and Piracy in the Soil

Iron is essential for the growth of virtually all organisms, but in the environment, soluble iron is a scarce commodity. Consequently, there is fierce competition between bacteria and fungi for the available iron. Microbes acquire iron through the release of small molecule siderophores. These specialized metabolites are used to scavenge iron: they bind Fe(III), and are recognized by receptors on the bacterial/fungal surface. The Fe-siderophore complex may then be actively transported into the cell (most common in bacteria), or the iron molecule may be selectively taken up (more common in fungi) (Saha et al. 2013). Many soil bacteria can recognize and take-up iron-bound siderophores produced by other species in a process known as siderophore-piracy.

When plated next the bacterium *Amycolatopsis sp*. AA4*, S. coelicolor* fails to develop aerial hyphae. This change in development is due to siderophore-piracy: *Amycolatopsis sp.* AA4 preferentially takes up the siderophore desferrioxamine produced by *S. coelicolor,* and consequently *S. coelicolor* fails to complete its aerial developmental program, as this is a highly iron-dependent process (Traxler et al. 2012). Notably, *Amycolatopsis* downregulates the expression of its native siderophore biosynthetic genes when growing next to *S. coelicolor*, which likely provides a strong competitive advantage, given that siderophore biosynthesis is an energetically expensive process (Traxler et al. 2012). In addition to desferrioxamine, *S. coelicolor* produces two other siderophores—coelichelin and coelibactin (Barona-Gomez et al. 2006). It is likely that different species have evolved the ability to use a variety of siderophores, and thus have the capacity to switch their iron-uptake strategies to minimize the effects of siderophore piracy by their neighbours (Barona-Gomez et al. 2006). More recent work has expanded the idea of siderophore-piracy to inter-kingdom interactions. Growth of a mutant *S. coelicolor* strain lacking the ability to produce desferrioxamine can be restored when grown near two different fungi—a *Penicillium* species and *Engyodontium album*, perhaps through its own act of stealing fungal siderophores (Arias et al. 2015).

How cells determine which iron-acquisition systems to activate is not fully understood. However, in multiple *Streptomyces* species, there are asRNAs expressed opposite both siderophore biosynthetic- and uptake-associated genes (Moody et al. 2013). For example, within the coelichelin biosynthetic cluster of *S. coelicolor,* a cutoRNA arises through the convergent transcription of *cchJ*, a putative coelichelin transporter, and *cchI* a coelichelin biosynthetic enzyme (Fig. 5.4f) (Lautru et al. 2005), similar to the situation in the actinorhodin cluster, where synthesis and transport are connected (see Fig. 5.4a). Further work is required to determine if these asRNAs are modulated in response to signals from other species.

5.10 Conclusions and Outlook

Although we are only beginning to understand how ncRNAs affect *Streptomyces* specialized metabolite production, it is clear that these compounds can mediate a wide range of interactions with other bacteria, fungi, insects and plants. Given the heterogeneous nature of the soil, it is not surprising that specialized metabolites appear to be central to the evolution of relationships between streptomycetes and other organisms. We have described a number of examples of *Streptomyces* inter-kingdom interactions mediated by specialized metabolites; in each case, asRNAs are associated with the corresponding biosynthetic pathways. These asRNAs have the potential to contribute to the regulation/production of the associated compound, possibly providing a more effective means of modulating gene expression than proteins, or perhaps simply providing a means of fine-tuning gene expression. Several described asRNAs are associated with specialized metabolite exporters, and in these cases, it is conceivable that asRNAs could modulate the levels of specialized metabolite secretion, and in doing so, could dictate whether the compound is secreted at low levels, when it might function as a signalling molecule, or at higher levels, where it may act as a weapon.

Since the majority of *Streptomyces* biosynthetic clusters are not expressed under laboratory conditions, it is impossible to predict how broadly asRNAs, and ncRNAs in general, will feature in the regulation of specialized metabolite production and inter-kingdom interactions. We anticipate that further studies of *Streptomyces* under more natural conditions will reveal widespread roles for specialized metabolites in the cooperation and competition with other organisms in its environment, and will reveal novel roles for asRNAs and other types of ncRNAs in the activation and regulation of specialized metabolite production.

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