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REVIEW

Avoidance of suicide in antibiotic-producing microbes

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Abstract Many microbes synthesize potentially autotoxic antibiotics, mainly as secondary metabolites, against which they need to protect themselves. This is done in various ways, ranging from target-based strategies (i.e. modification of normal drug receptors or de novo synthesis of the latter in drug-resistant form) to the adoption of metabolic shielding and/or efflux strategies that prevent drug–target interactions. These self-defence mechanisms have been studied most intensively in antibiotic-producing prokaryotes, of which the most prolific are the actinomycetes. Only a few documented examples pertain to lower eukaryotes while higher organisms have hardly been addressed in this context. Thus, many plant alkaloids, variously described as herbivore repellents or nitrogen excretion devices, are truly antibiotics—even if toxic to humans. As just one example, bulbs of *Narcissus* spp. (including the King Alfred daffodil) accumulate narciclasine that binds to the larger subunit of the eukaryotic ribosome and inhibits peptide bond formation. However, ribosomes in the *Amaryllidaceae* have not been tested for possible resistance to narciclasine and other alkaloids. Clearly, the prevalence of suicide avoidance is likely to extend well beyond the remit of the present article.

Keywords Antibiotic production · Antibiotic resistance · Antibiotic · Secondary metabolism · Natural products

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Introduction

Just as chemotherapy is an exercise in comparative biochemistry, so too is selective antibiotic resistance. A drug does not act unless it binds to something and if the target for a given drug is absent from certain cell types the latter will resist that drug. In some cases the basis of selective toxicity is obvious and, in a sense, trivial although certainly not in the practical context. Penicillin is antibacterial because it affects the biosynthesis of bacterial cell walls, which are chemically quite different from those of the fungi that produce penicillin. On the other hand, synthesis of penicillin-related compounds by bacteria poses the much more interesting problem of self-resistance to potentially autotoxic compounds. We have previously reviewed this topic separately [30, 37], since when the subject matter has expanded enormously and technology available to address it has developed beyond expectation. This article is not meant to be a directory. In attempting to provide a more narrative approach, invidious choices have been faced and we have knowingly omitted material that others might have included.

Antibiotics are ‘secondary metabolites’ that are not essential for normal growth and replication of the producing organisms. Secondary metabolism is practised by many (all?) cell types and generates an extravagant range of products, of which only a minority are antibiotics. Most antibiotics used in human medicine are microbial secondary metabolites, mainly produced by actinomycetes. As the latter organisms are quite receptive to genetical and biochemical manipulation, interest in self-resistance has been somewhat biased towards them.

‘Resistance’ is a purely relative term that does not equate to insensitivity. Early in batch culture, growth of antibiotic producers can commonly be inhibited by addition of the respective autogenous drugs at concentrations much lower

than those that otherwise accumulate later [37]. Such observations reflect a capacity for drug production (idiophase) that extends beyond the period of normal growth (trophophase), so that continued antibiotic production at later times involves non-growing cells. However, with the same organisms, in alternative media or under different growth conditions, idiophase and trophophase can overlap. In some cases, resistance of the producer increases as fermentation progresses; other producers benefit from constitutive resistance. Hence, the notion that antibiotic-producing organisms are sensitive to their products during trophophase but resistant during idiophase is not tenable as a generalization, although it evidently applies in quite a few cases. The degree of overlap between trophophase and idiophase reflects the extent to which idiophase is delayed via nutritional and environmental parameters. When growth can proceed optimally, secondary metabolism is suppressed and remains so for as long as rapidly utilizable nutrients are available in balanced amounts. Idiophase begins when the growth rate declines due to nutrient depletion or other environmental changes. Depending on the conditions, which in batch culture are not constant, synthesis of secondary metabolites (including antibiotics) can lag behind, then accompany, and eventually supersede biomass production.

Linkage between antibiotic production and resistance has an important history, not least because concurrent loss of both, by mutants, has commonly been observed in the drug industry. Perhaps it 'stood to reason' that enhanced production of a toxic substance might be facilitated by enhanced resistance of the producer—although just how cause and effect were supposed to operate was not always clear. But in any event, selection for increased resistance to the product did result in higher yields in empirical strain-improvement programs. With hindsight, the likelihood of this happening in any given organism probably depended largely on gene organization. Antibiotic-biosynthetic genes are typically arranged in clusters that also include resistance determinants and regulators. Among the latter are transcriptional activators that turn on biosynthesis genes and, sometimes, also resistance determinants. Crucially, enhanced expression of pathway-specific activator genes typically increases antibiotic production [159], revealing that the activator proteins are not normally present at saturating levels. Promoter mutations in such regulatory genes would commonly have enhanced antibiotic production, and selection for enhanced resistance would have identified those strains in which resistance genes just happened to be cotranscribed with 'enhanced' regulators.

Antibiotic resistance ultimately involves reduction or prevention of drug–target interactions; in the producing organisms, this can be achieved in various ways. On the one hand, when access of endogenous drug molecules to their ostensible target(s) cannot be prevented, the latter might be specifically designed in drug-resistant form. A totally different resistance strategy involves denial of drug-access to targets that remain sensitive. This can be done via metabolic shielding whereby active drug is produced only during (or after) export from the producer. Alternatively, resistance might depend simply upon efficient drug efflux or might involve factors that bind directly to the target or sequester the drug.

Resistance to intracellular antibiotics during their biosynthesis is not the only potential problem faced by producers. The latter might also have to cope with previously exported drug or with exogenous material perhaps emanating from siblings. Presumably, extracellular drug would not normally have unimpeded re-entry into the producer, especially if the latter were dependent on efflux for resistance. That would generate a futile cycle that might be energetically unsustainable. But even so, there is no doubt that some antibiotics can get back into their respective producers, at least to some extent. One conclusion from this review will be that many antibiotic producers use resistance mechanisms in combination and that the contributions of efflux to resistance, while sometimes difficult to quantitate, are pervasive.

Resistance involving antibiotic target sites

Antibiotics bind to specific receptors located inside sensitive cells or at their surfaces and the number of such targets is finite. For example, bacterial cells contain about 10^5 ribosomes at most. Accordingly, as enzyme turnover numbers in the range 10^3–10^4 per sec are commonplace, resistance phenotypes that result from enzymic modification of ribosomes are normally expressed maximally, even with poorly expressed resistance genes. In contrast, when resistance depends on drug inactivation, there might be a direct relationship between the amount of drug-inactivating enzyme produced and the level of resistance conferred. Resistance due to target modification is widespread and commonly involves ribosomes. These are assembled from the products of wild-type 'housekeeping' genes and resistance is due to covalent modification of their RNA component(s) by the products of discrete (non-ribosomal) genes. In this way, high levels of resistance (including, in some cases, insensitivity) can be achieved. However, target-based resistance does not necessarily involve target modification, but can arise from de novo synthesis of a drug-immune version of the target, for example, RNA polymerase. In this scenario, housekeeping genes determine resistance. Yet again, other producing organisms (e.g. some that make inhibitors of DNA gyrase) utilize de novo synthesis of both sensitive and resistant versions of the target. And finally in this overview, resistance can depend

on 'target protection' involving proteins that bind to and occlude intrinsically sensitive targets and/or displace pre-bound drug from their targets. For example, such proteins can protect ribosomes from inhibition by tetracycline.

Ribosomal targets

The ribosome is a prime target for antibiotic action and most drugs that inhibit protein synthesis do so by binding reversibly to specific sites located on one or other of the two ribosomal subunits. The ribosome is an RNA enzyme and antibiotics interfere with ribosomal function principally by binding to ribosomal RNA (rRNA). Such binding can be prevented by site-specific methylation of rRNA and this tactic is utilized (constitutively or inducibly) as a highly efficient self-resistance mechanism by various organisms that produce ribosome inhibitors (Table 1), but not by all. Ribosome-based resistance has not been encountered in the producers of chloramphenicol, streptomycin, neomycin, puromycin or tetracycline, for example.

Once antibiotic-resistant ribosomes have been detected in a given strain (either an antibiotic producer or a clone carrying DNA from a producer), the ribosomal component(s) responsible for resistance need to be identified. Ideally, this should involve reconstitution analysis in which components (RNA and protein) from sensitive and resistant ribosomes are used in crossover fashion to assemble chimeric particles that can be assessed for their drug response. In each case yet examined in detail (for technical reasons these all relate to prokaryotic ribosomes) self-resistance has been linked to ribosomal RNA (rRNA) rather than to proteins and the critical parameter is post-transcriptional methylation. To establish this model, causal connections had to be made linking resistance to the action of specific methyltransferases present in the producing organisms. In some cases, this could be done in vitro in a single step with purified enzymes via direct methylation of intact ribosomal subunits. However, other resistance methylases were active only on free rRNA. This necessitated further reconstitution analysis before the drug response of ribosomes containing in vitro methylated rRNA could be assessed. Alternatively, and less arduously, clone-specific methylase activities (associated with suitably short DNA fragments) that conferred specific resistance phenotypes were taken to establish cause and effect.

In various antibiotic-producing organisms, resistance has been accounted for by the action of a single rRNA methylase acting at a single site characteristic of a given phenotype (Table 2). This holds even for complex patterns of resistance, e.g. resistance to macrolides, lincosaminides and streptogramin B (the 'MLS' resistance phenotype) as discussed below. However, there are exceptions. Discrete pairs of rRNA methylases act together (neither alone is optimal) to confer resistance to tylosin in *Streptomyces fradiae* and to avilamycin in *Streptomyces viridochromogenes*. Also, in *Streptomyces* (now *Streptoalloteichus*) *tenebrarius*, the combined activities of two rRNA methylases give resistance to a broad range of aminoglycosides, although these enzymes also act separately—each conferring resistance to specific drugs. This latter, extravagant strain also possesses two genes encoding aminoglycoside acetyltransferase activities that result in substrate inactivation. Additional comments, relating to these and other strains, follow.

Thiostrepton

Ribosomes in the thiostrepton producer *Streptomyces azureus* are totally resistant at all times to the action of thiostrepton and other related thiopeptides. This is due to a methylase (the product of *tsr*; Table 2) that acts on free 23S rRNA but not on intact 50S ribosomal subunits [29]. Monomethylation takes place on pentose at residue A-1067, generating 2′-*O*-methyladenosine, and ribosomes containing such methylated RNA do not bind thiostrepton [237]. Other thiostrepton producers (notably *Streptomyces laurentii*—the preferred industrial production strain) and actinomycetes that make other thiopeptides (including siomycin, nosiheptide and berninamycin; Table 2) possess similar methylase activity and harbour orthologues of *tsr* that also confer thiopeptide resistance. The obvious causal inference is presumably valid. Unexpectedly, however, the thiostrepton-biosynthetic gene cluster of *S. laurentii* lacks any obvious resistance determinant(s); not even candidate efflux genes are present [94, 118]. The *tsr* orthologue, *tsnR*, which confers resistance to thiostrepton when cloned in *Streptomyces lividans*, is located among ribosomal protein operons in a 'housekeeping' region of the *S. laurentii* genome [211].

Macrolides: general considerations

The 'MLS' phenotype, concurrent resistance to *m*acrolides (such as erythromycin), *l*incomycin and compounds related to *s*treptogramin B (of which there are many, including virginiamycin S and pristinamycin IA), was first encountered in Gram-positive clinical isolates (for a review, see [254]), but is also widespread among actinomycetes, including (but not restricted to) macrolide producers [56]. Because such resistance involves methylation of ribosomal RNA, the early MLS genes were designated '*erm*' (*e*rythromycin *r*ibosome *m*ethylation) and later, following sequence comparisons with actinobacterial genes, a standardized nomenclature for *erm* genes was established (Table 3). However, gene designations used in the original literature have been retained in the present article for convenience of cross-reference.

The products of *ermC* from staphylococcus and *ermE* from the erythromycin producer *Saccharopolyspora*

Table 1 Antibiotic resistance via modification of ribosomal target site

Antibiotic group	Antibiotic	Producer	References
Thiopeptide	Thiostrepton	*S. azureus*	[29]
	Thiostrepton	*S. hawaiiensis*	[235]
	Nosiheptide	*S. actuosus*	[32]
	Siomycin	*S. sioyaensis*	[235]
	Berninamycin	*S. bernensis*	[236]
	Sporangiomycin	*P. parontospora* subsp. *antibiotica*	[235]
Macrolide	Erythromycin	*Sac. erythraea*	[225]
	Tylosin	*S. fradiae*	[13]
	Carbomycin[a]	*S. thermotolerans*	[265]
	Spiramycin	*S. ambofaciens*	[175]
	Mycinamicin	*M. griseorubida*	[85]
	Midecamycin	*S. mycarofaciens*	[72]
Lincosaminide	Lincomycin	*S. lincolnensis*	[268]
	Celesticetin	*S. caelestis*	[21]
Orthosomycin	Avilamycin	*S. viridochromogenes*	[255]
Aminoglycoside	Kanamycin	*S. kanamyceticus*	[155]
	Nebramycin[b]	*S. tenebrarius*	[263]
	Gentamicin	*M. purpurea*	[179]
	Sisomicin	*M. rosea: M. inyoensis*	[135]
	G-52	*M. zionensis*	[135]
	G-418	*M. rhodorangea*	[135]
	Verdamicin	*M. grisea*	[135]
	Sagamicin	*M. sagamiensis*	[135]
Pseudodisaccharide	Istamycin	*S. tenjimariensis*	[161, 262]
	Sporaricin	*Sac. hirsuta*	[79, 161]
	Sannamycin	*S. sannanensis*	[161]
	Fortimicin A[c]	*M. olivasterospora*	[161]
	Dactimicin	*D. matsuzakiense*	[161]
	SF-2052	*Micromonospora* sp SF-2098	[161]
Aminocyclopentitol	Pactamycin	*S. pactum*	[22]
Trichothecene	Trichothecin	*T. roseum*	[83]
	T-2 toxin	*My. verrucaria*	[78]

S., Streptomyces; P., Planomonospora; Sac., Saccharopolyspora; M., Micromonospora; D., Dactylosporangium; T., Trichothecium; My., Myrothecium

[a] Synonym, magnamycin

[b] Nebramycin complex includes tobramycin plus apramycin

[c] Synonym, astromicin

erythraea (formerly *Streptomyces erythraeus*) each generate a single residue of N^6,N^6-dimethyladenine at position 2058 within 23S rRNA [209] whereas other *erm* gene products monomethylate that site. Accordingly, there are two MLS phenotypes. The MLS type I mechanism (N^6-monomethylation of A-2058) confers high-level resistance to lincomycin with much lesser resistance to macrolides and streptogramin B whereas N^6,N^6-dimethylation of A-2058 (the type II mechanism) generates high-level resistance to all MLS antibiotics [176]. Many, but not all, macrolide producers harbour at least one *erm*-type gene although the stoichiometry of methylation (and, therefore, the detailed phenotype) cannot be predicted from *in silico* sequence comparisons and must therefore be determined directly. Curiously, ribosome-based resistance has not been encountered among the many producers of streptogramin-related drugs. Producers of lincomycin and related compounds are considered below.

Several macrolide producers that harbour *erm* resistance genes are listed in Tables 2 and 3. Type II MLS resistance is conferred constitutively by *ermE* in *Sac. erythraea* [65] but inducibly by *tlrA* (synonym *ermSF*) in the tylosin

Table 2 Self-resistance via methylation of ribosomal RNA

Antibiotic	Producer	Gene	Site 23S RNA	References
Thiostrepton	*S. azureus*	*tsr*	Ar-1067[a]	[237]
Thiostrepton	*S. laurentii*		Ar-1067[a]	[235]
Siomycin	*S. sioyaensis*		Ar-1067[a]	[235]
Nosiheptide	*S. actuosus*		Ar-1067[a]	[32]
Erythromycin	*Sac. erythraea*	*ermE*	A-2058[b]	[209]
Tylosin	*S. fradiae*	*tlrA*	A-2058[b]	[264]
		tlrD	A-2058	[266]
		tlrB	G-748	[122]
Spiramycin	*S. ambofaciens*	*srmA*	A-2058	[177]
		srmD	A-2058	[177]
Carbomycin	*S. thermotolerans*	*carB*	A-2058	[265]
Lincomycin	*S. lincolnensis*	*lmrB*	N/D	[268]
Celesticetin	*S. caelestis*	*clr*	A-2058	[21]
Avilamycin	*S. viridochromogenes*	*aviRa*	G-2535	[240]
		aviRb	Ur-2479[a]	[240]

Antibiotic	Producer	Gene	Site 16S RNA	References
Kanamycin	*S. kanamyceticus*	*kmr*	G-1405	[31]
Nebramycin complex	*S. tenebrarius*	*kgmB*	G-1405	[8, 31]
		kamB	A-1408	[199]
Gentamicin	*M. purpurea*	*grmA*	G-1405	[199]
G-52	*M. zionensis*	*sgm*	G-1405	[199]
Fortimicin A	*M. olivasterospora*	*fmrO*	G-1405[c]	[163]
Istamycin	*S. tenjimariensis*	*fmrT* (*kamA*)	A-1408	[8]
Sporaricin	*Sac. hirsuta*	*fmrH* (*kamC*)	A-1408	[79]
Pactamycin	*S. pactum*	*pct*	G-964	[7]

N/D not determined

[a] Pentose methylation; *Ar,* 2′-*O*-methyladenosine; *Ur*, 2′-*O*-methyluridine

[b] Dimethylation

[c] Site inferred

producer *S. fradiae* [264]. Among actinomycetes, type I MLS resistance appears more prevalent than type II and occurs constitutively due to *carB* in the carbomycin producer *Streptomyces thermotolerans* [265], and inducibly due to *srmA* and/or *srmD* in *Streptomyces ambofaciens* [177], the producer of spiramycin. Possession of multiple *erm*-type genes in individual actinomycetes is not rare and this point is re-addressed below.

Macrolide antibiotics bind to their target within a confined space, inside the tunnel that traverses the larger ribosomal subunit and through which the nascent peptide must pass. The drug molecule lies against the floor of the tunnel with the C-5 substituent sugar(s) pointing back towards the entrance, i.e. towards the site of peptide bond formation. This part of the tunnel is lined almost exclusively by 23S RNA with which macrolides make multiple interactions notably via their saccharidic moieties. In particular, residue A-2058 of 23S RNA forms hydrogen bonds with the 2′-OH of the sugar attached at C-5 of the macrolide lactone ring [71, 200] and substitution on either participant engenders macrolide resistance. This explains why N^6-methylation of A-2058 (and especially the more bulky dimethylation) inhibits ribosomal binding of macrolides and also why these drugs are inactivated by 2′-*O*-phosphorylation [158, 258] or 2′-*O*-glycosylation [106]. In some organisms, the latter macrolide glycosyltransferase (MGT) activity operates in tandem with Erm-type rRNA methylation as a ‘double resistance’ mechanism and MGT also features in the metabolic shielding strategy of the oleandomycin producer (discussed below). Macrolides like tylosin and spiramycin that have a disaccharidic substituent at the lactone C-5, make additional contacts with the tunnel wall via the sugar (mycarose) that is distal to the lactone ring [71]. Hence monomethylation at A-2058 confers lesser resistance to tylosin than to erythromycin. Although

Table 3 Revised nomenclature for MLS resistance determinants (data taken from [191])

Original gene name	Revised gene name	Protein	Organism
ermE	*erm*(E)	Erm(E)	*Saccharopolyspora erythraea*
tlrA (*ermSF*)	*erm*(S)	Erm(S)	*Streptomyces fradiae*
tlrD	*erm*(N)	Erm(N)	*Streptomyces fradiae*
srmA	*erm*(O)	Erm(O)	*Streptomyces ambofaciens*
srmD	*erm*(2)	Erm(2)	*Streptomyces ambofaciens*
carB	*erm*(H)	Erm(H)	*Streptomyces thermotolerans*
myrB	*erm*(W)	Erm(W)	*Micromonospora griseorubida*
mdmA	*erm*(I)	Erm(I)	*Streptomyces mycarofaciens*
lmrB	*erm*(U)	Erm(U)	*Streptomyces lincolnensis*
clr	Unclassified	Clr	*Streptomyces caelestis*

the lincomycin derivative clindamycin makes multiple hydrogen bonds with the tunnel wall, these predominantly involve A-2058 and even N^6-monomethylation of the latter causes high-level resistance [200].

Tylosin

In addition to the hydrogen bond interactions common to macrolides in general, tylosin makes idiosyncratic interactions with the ribosomal tunnel via a third sugar (mycinose) attached to the lactone ring at C-15, i.e. opposite the C-5 disaccharide. The mycinose moiety reaches across the tunnel and interacts with G-748 and A-752 of 23S RNA within the wall on the opposite side to A-2058, thereby enhancing the potency of tylosin and decreasing the efficacy of A-2058 monomethylation as a tylosin resistance mechanism. To achieve tylosin resistance requires methylation of 23S rRNA on both sides of the tunnel [122] or dimethylation at A-2058. Both of these options are available to the tylosin producer *S. fradiae*, in which resistance arises from interplay between the products of four genes (*tlrA*, *tlrB*, *tlrC*, *tlrD*), the last three of which lie within the tylosin-biosynthetic cluster. One route to effective tylosin resistance involves constitutive monomethylation of A-2058 by the product of *tlrD* [266] in concert with inducible monomethylation of G-748 by TlrB [122]. These adjacent RNA methylations on either side of the tunnel sterically block interaction of the mycaminose and mycinose moieties of tylosin with the tunnel wall and this generates a significant level of resistance [122], especially alongside induced expression of *tlrC* that encodes a deduced ABC transporter component [193]. An alternative, or even concurrent, route to self-protection for *S. fradiae* is via induction of *tlrA*, the product of which dimethylates A-2058 or completes that process following monomethylation by TlrD [264]. Perhaps the products of *tlrD, tlrB* and then *tlrA* come into play sequentially due to possible differences in the induction specificities of *tlrB* and *tlrA* [122]. In any event, their concerted action renders *S. fradiae* highly resistant to its product.

Spiramycin

Inducible resistance to spiramycin in the producer *S. ambofaciens* develops during the later stages of fermentation, or earlier when spiramycin is added to the growth medium [175]. This organism seemingly harbours a surfeit of resistance genes that, curiously, are present in pairs. These include two inducible *erm*-type I genes: *srmD* within the spiramycin-biosynthetic (*spi*) cluster and *srmA* that lies elsewhere [89] plus two genes that encode deduced ABC drug transporters. Again, one of the latter, *srmB* [203], is in the *spi* cluster whereas SrmC1-SrmC2 is encoded elsewhere [J.-L. Pernodet, personal communication]. Also outside the *spi* cluster, *srmA* is cotranscribed with *gimA*—a determinant of MGT activity [64]. Similar gene pairs are also found in other organisms (e.g. *S. lividans*) and make interesting resistance determinants as their products act in complementary fashion to perturb rRNA–macrolide interaction, as discussed above. Another MGT (GimB) that also inactivates spiramycin is present in *gimA*-disrupted *S. ambofaciens*, although its gene has not yet been identified [J.-L. Pernodet, personal communication], and within the *spi* cluster *orf29* is deduced to encode a secreted β-glucosidase. Such an enzyme might reactivate 2′-*O*-glycosylspiramycin, hypothetically generated by MGT activity that might be involved in spiramycin biosynthesis or resistance [89].

Like the *tsr* protein of *S. azureus*, the Erm methylases act on free 23S rRNA and not on intact ribosomes. Accordingly, inducible expression of an *erm*-type gene in an antibiotic producer would most effectively be triggered in advance of drug production. Otherwise, control of intracellular drug levels might be necessary to allow growth-dependent dilution of the sensitive ribosomal population. Drug efflux would be a likely participant in such a scenario.

Lincosaminides

The celesticetin producer *Streptomyces caelestis* harbours a constitutive resistance gene (*clr*) that encodes a classical *erm* type I resistance mechanism [21]. However, *clr* does not hybridize to the genome of *Streptomyces lincolnensis*, a lincomycin producer, even though this organism clearly utilizes lincomycin-resistant ribosomes and possesses a resistance determinant (*lmrB*) that resembles an *erm* gene *in silico* [268]. When *lmrB* was cloned in *S. lividans*, resistance was highly specific for lincomycin and did not even extend to its derivative clindamycin, i.e. *lmrB* did not confer a typical MLS phenotype. Accordingly, *lmrB* might not be an *erm* gene at all [268] even though it has been enrolled into the standardized *erm* classification (Table 3). The matter remains unresolved. Two other resistance determinants (*lmrA* and *lmrC*) have also been isolated from *S. lincolnensis* and shown to confer resistance when cloned in *S. lividans*. Like *lmrB*, these genes are present within the lincomycin-biosynthetic gene cluster. The deduced product of *lmrA* is a proton-dependent MFS drug antiporter [268] whereas LmrC is a hypothetical ABC transport component, similar to TlrC [178]. Drug efflux apparently makes a significant contribution to resistance in *S. lincolnensis*.

Avilamycin

Two genes, *aviRa* and *aviRb*, each encoding rRNA methyltransferase activity, were isolated from the avilamycin producer *Streptomyces viridochromogenes* Tü57, and each conferred resistance to avilamycin when expressed in

S. lividans. However, for maximal effect both genes had to be expressed concurrently—under which conditions the ribosomes became insensitive to avilamycin [255]. Purified AviRa and AviRb were active on free 23S rRNA from *Escherichia coli* (but not on ribosomal subunits) and generated N^1-methylG and 2′-*O*-methylU, at residues G-2535 and U-2479, respectively, (Table 2). Within the ribosome, G-2535 and U-2479 are about 10 Å apart, suggesting that they both lie within a single avilamycin-binding site that is fully occluded only when both nucleosides are methylated [240]. The combined action of these two gene products would clearly be sufficient for self-protection of the avilamycin producer.

In addition to *aviRa* and *aviRb*, the avilamycin-biosynthetic gene cluster [256] contains *aviABC1* and *aviABC2* that are deduced to encode two components of a single ABC transporter. However, when these genes were expressed in *S. lividans*, even from a strong promoter, only low level resistance was observed, perhaps suggesting that they encode drug efflux rather than resistance [255].

Aminoglycosides and Pseudodisaccharides

Ribosomal modification resulting in aminoglycoside resistance involves monomethylation of 16S RNA, within the 30S ribosomal subunit, at either of two sites: G-1405 or A-1408. Substitution occurs on ring nitrogen to generate 7-methylguanosine or 1-methyladenosine, respectively [8], and this blocks binding of specific drugs to the ribosome. Such resistance has recently been observed among clinical enterobacteria but was first characterized in actinomycetes that produce aminoglycosides.

Methylation at G-1405 of 16S rRNA confers high levels of resistance to kanamycin and gentamicin but not neomycin [8]. Hence, the resistance determinants are designated *kgm* (*k*anamycin-*g*entamicin resistance *m*ethylation). In contrast, methylation at A-1408 generates high-level resistance to the kanamycins and the novel aminoglycoside apramycin [8]. The genes responsible are therefore designated *kam* (*k*anamycin-*a*pramycin resistance *m*ethylation. The Kam mechanism does not give gentamicin resistance and the conferred level of resistance to (tetracyclic) neomycin or paromomycin is low. However, Kam strains are almost insensitive to dicyclic (e.g. neamine) and tricyclic (e.g. ribostamycin) members of the neomycin-paromomycin family.

A gene, *kgmA*, believed to originate from the gentamicin producer *Micromonospora purpurea* [238] was later shown to have been confused with *kgmB* from *S. tenebrarius* [207]. For further details, see [31]. Therefore, the original characterization of the Kgm resistance mechanism [8] had actually been done with the product of *kgmB*. Similar modification of residue G-1405 was detected in 16S rRNA from the kanamycin producer *Streptomyces kanamyceticus* after growth in production medium but not otherwise (A. A. D. Beauclerk and E. Cundliffe, unpublished data; reported in [31]) and also in *S. lividans* harbouring the *kmr* gene (also referred to as *kan*), originally isolated from *S. kanamyceticus* [155]. Thus, the Kmr protein is a Kgm methylase. Later, an authentic *M. purpurea* resistance gene (*grmA*) that also conferred the Kgm phenotype was isolated [93] and its product has recently been shown, securely, to act in identical fashion to KgmB, as has the product of *sgm* from *Micromonospora zionensis*, the sisomicin producer [199].

The Kam resistance mechanism was originally characterized [8] by using the product of *kamA* from *Streptomyces tenjimariensis* [208] and later [79] KamC from *Saccharopolyspora hirsuta* was also shown to modify A-1408 within 16S rRNA. Recently [199], it has been confirmed that A-1408 is the target for *KamB* from *S. tenebrarius* [207] and the *in silico* assignment of respective start codons in the deposited sequences of *kamB* and *kamC* was corrected to clarify their relationship to other *kam* genes [199].

When *kamA* was first isolated (as a kanamycin resistance determinant) from *S. tenjimariensis* [208], a possible role for that gene in self-protection could not be addressed as the autogenous drug istamycin (a member of the fortimicin group of pseudodisaccharides) was not available. The fortimicins (astromicins) are produced by actinomycetes from four genera that all contain fortimicin resistance (*fmr*) genes (Table 1). However, hybridization analysis clearly split the latter genes into two groups (related to *fmrT* or *fmrO*, respectively) even though they all confer cross-resistance to kanamycin [161]. The *fmrT*-related genes also encode low-level neomycin resistance whereas those related to *fmrO* give gentamicin resistance. Sequence analysis established that the *fmrT* group are related to *kam* genes and confirmed the identity of *fmrT* and *kamA* from *S. tenjimariensis* [162], whereas the *fmrO* group belong to the *kgm* family [163]. Evidently, *fmrT*-related and *fmrO*-like genes are responsible for self-protection in their natural hosts.

The Kgm and Kam methylases act on intact 30S ribosomal subunits and, when expressed inducibly, they can generate resistance phenotypes much more rapidly than do inducible methylases that act only on free rRNA. Genes of the *kgm* and *kam* families are typically present in the respective antibiotic-biosynthetic clusters. Thus, *grmA*, *kmr* and *kamA* (*fmrT*) are found within the gentamicin-, kanamycin- and istamycin-biosynthetic clusters of *M. purpurea* (now *M. echinospora*), *S. kanamyceticus* and *S. tenjimariensis*, respectively. In the nebramycin producer *S. tenebrarius*, *kamB* is present within the apramycin gene cluster and confers resistance to the entire nebramycin complex (apramycin plus derivatives of kanamycin B, including tobramycin) whereas, curiously, the cohabitant

tobramycin resistance determinant *kgmB* is absent from the tobramycin gene cluster. Whether or not the latter is superfluous for resistance in *S. tenebrarius* will not be clear until the patterns of expression of *kamB* and *kgmB* can be related to production of the respective antibiotics.

Non-ribosomal targets

Target RNA polymerase: Ansamycins, Streptolydigin

The major catalytic component of bacterial RNA polymerase is the β subunit (product of the *rpoB* gene) and this protein is the target for various antibiotics, including the ansamycins (rifamycin and its derivative rifampicin; streptovaricin; tolypomycin; geldanamycin) and also streptolydigin. Organisms that produce these compounds (Table 4) each harbour RNA polymerase that is intrinsically and specifically resistant to the respective autogenous drug [195]. Resistance is constitutive and there is no suggestion that the drug response of RNA polymerase is due to post-translational modification, nor has drug inactivation been detected in these producing organisms (J.A. Salas, personal communication). Hybridization and PCR analyses suggest that *Amycolatopsis mediterranei*, *Streptomyces lydicus* and *Streptomyces spectabilis* each harbour only a single *rpoB* gene and, interestingly, the rifamycin-biosynthetic (*rif*) gene cluster of *A. mediterranei* is located immediately alongside *rpoB* in what would otherwise be considered a 'housekeeping' region of the chromosome [53]. Within the *rif* cluster, *rifP* is deduced to encode a transmembrane protein of the MFS variety that might utilize the chemiosmotic proton gradient to drive drug export.

Target EF-Tu: Elfamycins, Pulvomycin, GE2270A

Elfamycin polyenes of the kirromycin (mocimycin) family including aurodox and efrotomycin, together with pulvomycin and thiazolyl peptides such as GE2270A, target the bacterial translation factor EF-Tu. The latter is one of the most abundant cytoplasmic proteins and many bacteria obtain it from two separate *tuf* genes (*tufA* and *tufB* in *E. coli*; *tuf1* and *tuf3* in *Streptomyces coelicolor*). Mutations conferring resistance to these antibiotics map to the *tuf* genes, although for kirromycin (and its derivatives) or pulvomycin the resistance phenotype is only expressed when each of the resident *tuf* genes is mutated, i.e. sensitivity to kirromycin or pulvomycin (but not GE2270A) is dominant over resistance. At least for kirromycin, this effect can be rationalized. By preventing dissociation of drug-sensitive EF-Tu from the ribosome during translation, kirromycin breaks the elongation cycle and blocks ribosomal migration along mRNA. Because multiple ribosomes translate individual mRNAs concurrently (the 'polyribosome' model), such stalling also blocks the progress of upstream ribosomes, including those that might be carrying kirromycin-resistant EF-Tu.

Table 4 Self-resistance involving non-ribosomal target sites

Antibiotic group	Antibiotic	Producer	Refractory target	References
Aminocoumarin	Novobiocin	*S. sphaeroides*	GyrB	[230]
	Coumermycin	*S. rishiriensis*	GyrB plus ParY	[201]
	Clorobiocin	*S. roseochromogenes*	GyrB plus ParY	[201]
Ansamycin	Rifamycin	*A. mediterranei*	RNA pol β	[195]
	Streptovaricin	*S. spectabilis*	RNA pol β	[195]
Acyltetramic acid	Streptolydigin	*S. lydicus*	RNA pol β	[195]
Cyclic peptide	α-Amanitin	*Amanita* spp.*	RNA pol II	[88]
Elfamycin	Kirrothricin	*S. cinnamoneus*	EF-Tu	[63]
	Efrotomycin	*N. lactamdurans*	EF-Tu	[63]
Thiazolylpeptide	GE2270A	*Pl. rosea*	EF-Tu	[150, 212]
Glycopeptide	Vancomycin	*A. orientalis*	Peptidoglycan	[131, 132]
Pseudomonic acid	Mupirocin	*Ps. fluorescens*	Ile-RS	[82]
Fatty acid amide	Cerulenin	*C. caerulens**	Fatty acyl synthase	[91]
Sesquiterpene	Pentalenolactone	*S. arenae*	GAPDH	[137]
Tripeptide	Phaseolotoxin	*Ps. syringae* pv. *phaseolicola*	OCT	[46, 216]
Cyclic peptide [polymyxin-type]	Colistin	*B. colistinus*	Cytoplasmic membrane	[206]
β-Methoxyacrylate	Strobilurin	*Stb. tenacellus**	Cytochrome bc_1	[104]

A., Amycolatopsis; *B., Bacillus*; *C., Cephalosporium*; *Pl., Planobispora*; *Ps., Pseudomonas*; *N., Nocardia*; *S., Streptomyces*; *Stb., Strobilurus*, *GAPDH* glyceraldehyde phosphate dehydrogenase, *OCT* ornithine carbamoyltransferase, *GyrB* DNA gyrase B subunit, *ParY* subunit of DNA topoisomerase IV

* Eukaryotic organisms

The dominance of kirromycin sensitivity and the genetic makeup of specific kirromycin producers combine to dictate possible modes of self-resistance. Organisms that employ drug-resistant EF-Tu each possess just a single ('housekeeping') *tuf* gene, whereas those with multiple *tuf* genes defend themselves in other ways. And in either event, *tuf* genes have not been encountered in the respective antibiotic-biosynthetic gene clusters. The kirrothricin producer *Streptomyces cinnamoneus* has a single *tuf* gene [25] and EF-Tu from this organism is resistant to kirrothricin, kirromycin and efrotomycin [63] but is sensitive to GE2270A [25]. Single *tuf* genes also confer resistance in the producers of efrotomycin and GE2270A (Table 4). Conversely, drug-sensitive EF-Tu is present in several elfamycin producers. These include the kirromycin producers *Streptomyces collinus* [63, 145] and *Streptomyces fradiae* Tü1222 [63] that each possess two *tuf* genes [166], and *Streptomyces diastatochromogenes* [63] that has only one [166]. Paradoxically, in the pulvomycin producer *Streptoverticillium mobaraense*, EF-Tu is sensitive to pulvomycin but resistant to the kirromycins [63]. The manner of self-defence in these various organisms is not clear and the respective antibiotic-biosynthetic gene clusters offer no obvious clues (such as candidate efflux systems etc.). A similar conclusion applies to the kirromycin producer *Streptomyces ramocissimus*. Remarkably, this organism has three *tuf* genes [245], one of which (*tuf3*) encodes a product (EF-Tu3) that is highly resistant to kirromycin, GE2270A and pulvomycin [167]. However, expression of *tuf3* is marginal at the best of times and was not detectable during, or immediately before, kirromycin production. By far the bulk of EF-Tu in this organism is encoded by *tuf1* and is drug-sensitive. That, and the dominance of kirromycin-sensitivity over resistance, means that EF-Tu3 cannot possibly play any significant role in resistance, so *S. ramocissimus* must ensure its own well-being in some other, uncharacterized way [167].

Target Glyceraldehyde Phosphate Dehydrogenase: Pentalenolactone

Although some antibiotic-producing organisms 'simply' produce a resistant version of the ostensible drug target, others can make alternative isoforms. Pentalenolactone irreversibly inactivates glyceraldehyde 3-phosphate dehydrogenase (GAPDH) via acylation at the active site, which poses an interesting dilemma for *Streptomyces arenae* given that production of the drug depends on carbohydrate utilization. To resolve this problem, *S. arenae* generates a pentalenolactone-insensitive version of GAPDH concurrently with the onset of antibiotic production [137]. This does not arise by modification of the pre-existing, drug-sensitive enzyme that is destroyed via proteolysis. The two GAPDH proteins are quite dissimilar and are encoded by discrete, differentially regulated genes within the *S. arenae* chromosome [55]. As the switch between GAPDH isozymes is also triggered when pentalenolactone is fed to *S. arenae* in non-production media [137], the resistance gene is apparently inducible by the drug.

Pentalenolactone is also produced by *Streptomyces avermitilis*, the same organism that makes avermectin. Within the cluster of pentalenolactone-biosynthetic (*ptl*) genes [228] lies *gap1* (*ptlK*) that encodes pentalenolactone-insensitive GAPDH activity, whereas a drug-sensitive isozyme is encoded by *gap2* elsewhere in the genome. Also encoded in the *ptl* cluster of *S. avermitilis* is a deduced MFS transporter that might reduce intracellular drug levels by facilitating efflux. Expression of the two *gap* genes is differentially regulated in *S. avermitilis*, as in *S. arenae*, and activity closely similar to that of *S. avermitilis* Gap1 has been expressed from a fragment of *S. arenae* DNA [54]. However, resistance comes at a price. Those features that prevent binding of pentalenolactone to the active site of GAPDH apparently reduce the catalytic efficiency of this vital glycolytic enzyme. Retention of the wild-type isoform to facilitate rapid growth during trophophase seems to be a price worth paying [54].

Target Type II DNA Topoisomerases: Aminocoumarins

Differential production of drug-target isoforms is also encountered in *Streptomyces sphaeroides*, producer of the aminocoumarin antibiotic novobiocin. Aminocoumarins act on the B subunit of bacterial DNA gyrase, a tetrameric A_2B_2 enzyme. This essential type II DNA topoisomerase uses the energy of ATP hydrolysis to introduce negative supercoils into closed circular duplex DNA (for a review, see [138]). Other enzymes, such as DNA topoisomerase I, act in the opposite sense and remove supercoils so that the superhelical density of DNA is dynamically controlled by the complementary activities of these and other DNA topoisomerases.

When *S. sphaeroides* was grown in the absence of antibiotic, only novobiocin-sensitive DNA gyrase activity was detectable, but resistant gyrase appeared when novobiocin was added to the medium [230]. This involved expression of a second *gyrB* gene (designated $gyrB^R$) that confers high-level resistance to novobiocin [229]. After $gyrB^R$ had been found at one end of the novobiocin-biosynthetic (*nov*) gene cluster in *S. sphaeroides* [217], equivalent genes were observed in equivalent locations in other aminocoumarin producers, such as *Streptomyces rishiriensis* and *Streptomyces roseochromogenes* that produce clorobiocin (chlorobiocin) or coumermycin A_1, respectively [182, 253]. This was not altogether surprising, but to find those $gyrB^R$ orthologues in tandem with other

topoisomerase genes was entirely unexpected. Immediately downstream of *gyrB*^{R} in the clorobiocin- and coumermycin A_1-biosynthetic clusters (Table 4) were genes (designated *parY*^{R}) encoding subunits of topoisomerase IV, an enzyme not previously encountered in actinobacteria [201]. This enzyme, like DNA gyrase, is a tetrameric type II topoisomerase comprising one subunit that cleaves and reseals DNA plus another (ParY in the *Streptomyces* enzyme) that resembles GyrB in coupling such activities to ATP hydrolysis. Unlike gyrase, which introduces supercoils into DNA circles, topoisomerase IV has a specific role in decatenation and relaxation of daughter DNA circles following replication. When cloned in *S. lividans*, *gyrB*^{R} and *parY*^{R} from *Streptomyces rishiriensis* each conferred resistance to novobiocin plus coumermycin A_1 as did *gyrB*^{R} from *S. sphaeroides* [202]. And significantly, a hybridization probe derived from *S. rishiriensis parY*^{R} found two targets in DNA from *S. rishiriensis* or *S. roseochromogenes* but only one in *S. sphaeroides* or *S. coelicolor* [202]. This incisive analysis revealed that in *Streptomyces* spp. both DNA gyrase and topoisomerase IV are normally sensitive to aminocoumarins with the former enzyme as the primary target. Although self-protection of the novobiocin producer can be achieved via synthesis of a drug-resistant form of gyrase B, the extreme potency of coumermycin A_1 and clorobiocin requires that producers of these drugs desensitize both targets. This they achieve by additional synthesis of a drug-resistant ParY isoform [201].

The *gyrB*^{R} promoter exhibits hair trigger activation in response to reduced DNA supercoiling—caused by, but not limited to, exposure of cells to novobiocin [230]. Although many promoters, especially those that control topoisomerase genes [144], respond to changes in DNA topology, the behaviour of the *gyrB*^{R} promoter is extreme by any standards. Thus, during early stages of novobiocin production in *S. sphaeroides*, it is proposed that the slightest inhibition of DNA gyrase activity causes a transient reduction in genomic supercoiling sufficient to turn on (sic) expression of *gyrB*^{R}. Formation of drug-resistant gyrase then restores the relative levels of opposing topoisomerase activities and allows continued growth in the presence of novobiocin [230]. In *S. rishiriensis* and *S. roseochromogenes*, where *gyrB*^{R} and *parY*^{R} are probably cotranscribed, resistance to coumermycin A_1 and clorobiocin is presumably regulated likewise.

Similarities between the aminocoumarin gene clusters probably do not extend to drug efflux systems. In *S. sphaeroides*, *novA* is deduced to encode an ABC drug transporter that could derive energy from ATP hydrolysis, whereas the deduced product of *couR5* in *S. rishiriensis* is an MFS protein that would likely utilize the chemiosmotic proton motive force [202]. When expressed in *S. lividans*, *novA* and *couR5* each conferred resistance to novobiocin plus coumermycin A_1, albeit at low levels. Other low-level determinants of novobiocin resistance, not represented by *gyrB*^{R} or *novA*, have been isolated from *Streptomyces niveus* (*S. sphaeroides*) but their products were not characterized [147].

Target Cell Wall-Cell Membrane: Glycopeptides

Vancomycin and other glycopeptides bind to the *C*-terminal D-Ala-D-Ala sequence of the stem pentapeptides of nascent peptidoglycan and thereby disrupt bacterial cell wall synthesis. To achieve this, the drugs gain access to their targets (the membrane-associated lipid II intermediate and multiple peptidoglycan growth points) from outside the cell. Resistance to glycopeptides involves synthesis of peptidoglycan precursors with altered stems that terminate in D-Ala-D-Lactate (D-Ala-D-Lac) to which glycopeptides bind with greatly reduced affinity. Three enzymic activities are required for glycopeptide resistance and, in clinical vancomycin-resistant enterococci, are encoded by *vanHAX*. First, pyruvate is converted to D-Lactate by VanH, then (crucially) VanA produces D-Ala-D-Lac alongside ongoing synthesis of D-Ala-D-Ala, while VanX specifically cleaves the latter dipeptide. Adventitious substrate flexibility then allows MurF (the enzyme that normally adds D-Ala-D-Ala) to attach D-Ala-D-Lac to the peptidoglycan precursor, UDP-*N*-acetylmuramyl-tripeptide, to generate an abnormal stem. The *vanHAX* genes are encountered on transposon Tn*1546* in enterococci and an equivalent resistance mechanism (in which VanB mimics VanA) is encoded on other mobile elements (for a review, see [5]).

Orthologues of *vanHAX* are present in glycopeptide producers and probably represent the original source of the clinical resistance determinants [132]. When a PCR strategy was used to clone D-Ala-D-Ala ligase (*ddl*) genes from the vancomycin producer *Amycolatopsis orientalis* and from glycopeptide-producing *Streptomyces toyocaensis*, the deduced protein products were highly similar to VanA (VanB) and when one of the PCR products (*ddlM* from *S. toyocaensis*) was expressed in *S. lividans*, D-Ala-D-Lac ligase activity was revealed [131]. In summary, two *ddl* genes were found in *S. toyocaensis*. The product of one of these, 'Ddl', was detected during mid-exponential growth phase and synthesized D-Ala-D-Ala exclusively, whereas the other enzyme (DdlM) generated D-Ala-D-Lac preferentially but not exclusively [133]. Significantly, DdlM was produced only during stationary phase or when the autogenous glycopeptide (A47934) was added to the growth medium, suggesting that *ddlM* might be inducible by glycopeptide(s) and that *S. toyocaensis* might switch between different modes of peptidoglycan formation. Sequencing of the A47934-biosynthetic gene cluster of *S.toyocaensis* [183] revealed orthologues of *vanHAX* as a presumptive

operon ($vanH_{st}$, $vanA_{st}$ (*ddlM*), $vanX_{st}$) located immediately adjacent to a deduced *murF* orthologue (designated *murX*). It is tempting to suggest that the latter might function as a D-Ala-D-Lac-adding enzyme. Quite how many other glycopeptide producers also utilize *vanHAX* orthologues is unclear. They are certainly present, alongside a *murF* orthologue, in the teicoplanin gene cluster of *Actinoplanes teichomyceticus* [117, 213] but not in the corresponding clusters in producers of the dalbavancin precursor A40926 [214], balhimycin [171, 189] or chloroeremomycin [243]. The latter observation is interesting as a *vanHAX* probe found hybridization targets in the genomes of all glycopeptide producers tested, including the chloroeremomycin producer *Amycolatopsis orientalis* [132]. The A40926 and balhimycin clusters each include a deduced orthologue of *vanY* [214]. The product of the latter gene is a D, D-carboxypeptidase that removes the *C*-terminal D-Ala residue from peptidoglycan pentapeptide stems, thereby destroying the glycopeptide target sequence in peptidoglycan units that escape the VanA (VanB) resistance mechanism. As *vanY* contributes significantly to glycopeptide resistance in enterococci, it might also do so in glycopeptide producers, regardless of whether they also possess *vanHAX* genes. Additionally, each of the aforementioned glycopeptide-biosynthetic gene clusters contains at least one *orf* that is deduced to encode an ABC drug transporter.

Target Ornithine Carbamoyltransferase: Phaseolotoxin

Phaseolotoxin, produced by *Pseudomonas syringae* pv. *phaseolicola*, causes halo blight in bean (*Phaseolus vulgaris* L.). The toxin is a modified tripeptide (ornithine*–alanine–homoarginine) that displays modest, but significant, inhibition of ornithine carbamoyltransferase (OCT) from whatever source. Phaseolotoxin inhibits OCT reversibly in vitro but *in planta* its effects are devastating as cleavage by non-specific host peptidase(s) liberates the toxic 'warhead' N^{δ}-(*N'*-sulfodiaminophosphinyl)-L-ornithine, 'PSorn' [148, 151]. The latter inhibits OCT irreversibly. Self-protection in the phaseolotoxin producer involves synthesis of a resistant version of OCT (designated ROCT) concurrently with toxin production, typically at 18–20°C [46]. In cells grown at 28–30°C, phaseolotoxin is not produced and OCT remains fully sensitive [216]. Two genes encoding OCT activity, *argF* and *argK*, are present in the producer [170] and one of them (*argK*, encoding ROCT) is located within the phaseolotoxin-biosynthetic cluster [1]. According to one model [126], temperature downshift is sensed in *P. syringae* pv. *phaseolicola* by an unknown mechanism that inactivates a transcriptional repressor, R_1. This allows expression of toxin-biosynthetic genes, leading to synthesis of the tripeptide and the *N'*-sulfodiaminophosphinyl moiety of PSorn. The latter (inorganic) moiety is proposed to inactivate another repressor, R_2, thereby derepressing *argK*. Thus, although temperature control of *argK* is indirect, the onset of resistance and toxin production are nevertheless coupled [126, 194].

Later in this article, discussion centres on self-resistance to tabtoxin in *Pseudomonas syringae* subsp. "*tabaci*". There again, a pretoxin is activated by proteolysis to release a phytotoxic amino acid analogue. However, that producer does not utilize a refractory version of the target enzyme but employs, instead, a conceptually different resistance strategy, namely, metabolic shielding.

Target protection

Before addressing resistance mechanisms that are not target-based, an intriguing 'hybrid' strategy is considered, whereby ribosomes can be protected from tetracycline in the absence of drug inactivation or sequestration.

Tetracycline

Two tetracycline-resistance determinants (formerly *tetA* and *tetB*; now *otrA* and *otrB*) were isolated from the oxytetracycline producer *Streptomyces rimosus* and expressed in *Streptomyces griseus* [160]. Both genes were inducible by tetracycline but whereas protein synthesis was resistant to the drug in extracts of *otrA* strains, expression of *otrB* was associated with decreased accumulation of exogenous tetracycline (more of this, later). The former character was associated with crude ribosomes that became sensitive to the drug when washed with salt, suggesting the involvement of a non-ribosomal factor in resistance [160]. Similarly, in extracts of *Streptococcus faecalis* harbouring plasmid-borne *tetM*, protein synthesis was found to be tetracycline-resistant [17]. It soon became clear that the deduced products of streptococcal *tetM* [134] and *tetO* from *Campylobacter* [129] were related and resembled the bacterial translation elongation factors EF-Tu and EF-G [198]. As tetracycline inhibits protein synthesis by preventing the binding of aminoacyl-tRNA to ribosomes (a process catalyzed by EF-Tu), this prompted plausible speculation that the TetM and TetO proteins (to which OtrA is also related [42]) might mediate tetracycline resistance by acting as drug-resistant translation factors. However, TetM does not act in that manner [18] nor does it bind tetracycline. Rather, TetM binds to ribosomes and displaces pre-bound tetracycline [18]. TetO behaves similarly and, together with TetM and OtrA, represents a family of 'Ribosomal Protection Proteins' (RPPs; for a review, see [27]). In their presence, tetracycline does not inhibit ribosomal function. The RRPs are widespread among bacteria, many are encoded on mobile genetic elements, and their genes plausibly originated in tetracycline-producing

actinomycetes [190]. In the oxytetracycline producer *S. rimosus*, self-protection via OtrA is supplemented by another non-ribosomal resistance mechanism involving OtrB.

The product of *S. rimosus* (*otrB*) is related to TetA, the 'resistance protein' (drug-carrier component) of the archetypal tetracycline efflux pump encoded on Tn*10* in *E. coli* [77]. This and other Tet pumps comprise two components: the substrate carrier (TetA) plus a transcriptional regulator (TetR) and these are typically encoded by divergent genes, for example, *otrB-otrR* in *S. rimosus* and *ctc05-ctcR* in *Streptomyces aureofaciens*, producer of chlortetracycline [115, 269]. The TetA carriers are integral membrane proteins, with multiple (12–14) membrane-spanning domains, that act in antiporter mode and derive energy from the chemiosmotic proton motive force. The TetR component is a transcriptional repressor, with a helix-turn-helix DNA-binding motif, that negatively controls the [*tetR-tetA*] gene pair by binding to divergent promoters within the intergenic DNA. Tetracycline induces the TetA system by classical derepression; at sub-inhibitory concentrations, the drug binds to TetR and releases it from target DNA. Transcription then proceeds from both promoters without further activation to generate TetA (and, with it, resistance) plus more TetR. When TetA reduces the intracellular drug concentration below some critical level, TetR eventually returns the system to the basal repressed state. In tetracycline producers, such a mechanism would presumably operate with the novel feature of continued drug production that would prolong the expression of resistance.

Antibiotic-inactivating enzymes in producers

Enzymes that inactivate the respective autogenous drugs might allow antibiotic producers to cope with previously exported drug, or material emanating from siblings, that might gain access into the producing cell. Alternatively (especially in the absence of target-based resistance), such enzymes might ensure that active drug molecules are not produced intracellularly. A selection of antibiotic-inactivating enzymes encountered in producers is presented in Table 5. There are more—perhaps lots more [35]. Although some of these activities were first observed many years ago, most are still curiosities and their contributions to the well-being of antibiotic-producing organisms remain enigmatic. Those in aminoglycoside producers pose particularly interesting questions.

Aminoglycosides

Bacteria that produce aminoglycosides of the neomycin family (including paromomycin, lividomycin, ribostamycin and butirosin) do not possess drug-resistant ribosomes. Rather, they all display aminoglycoside phosphotransferase, APH(3′), and aminoglycoside acetyltransferase, AAC(3), activities while the neomycin producer *Streptomyces fradiae* additionally harbours AAC(2′). When genes encoding APH(3′) or AAC(3) were isolated from *S. fradiae* (or from the paromomycin producer *Streptomyces rimosus* forma *paromomycinus*) and introduced separately into *S. lividans*, they each conferred low-level resistance to neomycin or paromomycin, whereas high-level resistance was observed when such *aph* and *aac* genes were coexpressed [172, 234]. The implications of these findings are unclear, not least because neomycin and paromomycin are inactivated by 3′-phosphorylation or by 3-acetylation. Perhaps doubly modified drug was produced in those studies and maybe it somehow blocked uptake of exogenous drug. In any event, emerging details of the paromomycin-neomycin biosynthetic pathway do not imply any role for APH(3′) or AAC(3) in that context. Equally obscure, is the role of ribostamycin carboxymethylation in *Streptomyces ribosidificus* [103].

The kanamycin producer *S. kanamyceticus* harbours AAC(6′) activity [9]. The gene encoding that enzyme, variously designated *kanM* [95] or *kanA* (accession number AJ628422.2), is present in the kanamycin-biosynthetic cluster and confers resistance when cloned in *S. lividans* [136]. Moreover, kanamycin yields were significantly enhanced when extra copies of this gene were introduced into *S. kanamyceticus* [28]. Nevertheless, the role of kanamycin acetyltransferase activity in the producer is unclear. As discussed earlier in this article, *S. kanamyceticus* is highly resistant to kanamycin via inducible methylation of ribosomal RNA, which might obviate a role for AAC(6′) in self-resistance. Similar considerations pertain to the synthesis of nebramycin, a complex of kanamycin B derivatives (notably tobramycin) plus apramycin. As *S. tenebrarius* is highly resistant to its products via two different rRNA methylation mechanisms [207], the additional presence of AAC(6′) and AAC(2′) activities in that strain [263] remains enigmatic.

Antibiotic-modifying enzymes specific for the autogenous drugs have not been characterized in *Micromonospora* spp. that produce the gentamicins. Such organisms achieve self-protection via methylation of rRNA.

Analysis of aminoglycoside-biosynthetic gene clusters suggests that producers of the neomycin-paromomycin family might use ABC transporters for drug efflux, while the kanamycin family and the gentamicins are perhaps exported via MFS-mediated antiport. Whether aminoglycoside-inactivating enzymes generate the exported forms of any of these drugs remains unclear.

Table 5 Antibiotic-modifying enzymes in the producing microorganisms

Antibiotic	Producer	Enzyme(s)	References.
Neomycin group aminoglycosides			
Neomycin	*S. fradiae*	APH(3′), AAC(3), AAC(2′)	[9, 34, 81]
Paromomycin	*S. rimosus* forma *paromomycinus*	APH(3′), AAC(3)	[34, 41]
Lividomycin	*S. lividus*	APH(3′), AAC(3)	[34]
Ribostamycin	*S. ribosidificus*	APH(3′), AAC(3)	[41, 102]
Butirosin	*B. circulans*	APH(3′), AAC(3)	[34, 41]
Kanamycin group aminoglycosides			
Kanamycin	*S. kanamyceticus*	AAC(6′)	[9]
Nebramycin	*S. tenebrarius*	AAC(6′), AAC(2′)	[33, 263]
Streptomycin	*S. griseus*	SPH(6), SPH(3″)	[146, 156]
	S. bikiniensis		
Spectinomycin	*S. flavopersicus*	Phosphotransferase	[127]
Hygromycin B	*S. hygroscopicus*	HPH	[111]
Puromycin	*S. alboniger*	PAC	[173]
Chloramphenicol	*S. venezuelae* ISP5230	CPT	[153]
		Hydrolase	[154]
Tuberactinomycins			
Viomycin	*S. vinaceus*	VPH	[210]
Capreomycin	*S. capreolus*	CPH	[210]
		CAC	[210]
Streptothricin	*Streptomyces* sp V-13-1	STAT	[92]
	S. lavendulae	STAT	
Nourseothricin	*S. noursei*	NAT	[73]
Macrolides			
Oleandomycin	*S. antibioticus*	MGT	[246]
(Neo)methymycin	*S. venezuelae* ATCC 15439		[270]
Fosfomycin	*S. wedmorensis*	Phosphotransferase	[108]
	Ps. syringae PB-5123	Phosphotransferase	[61]
Bialaphos	*S. hygroscopicus*	(D)PAT	[84]
Tabtoxin	*Ps. syringae* pv. *tabaci*	TβL acetyltransferase	[3]
		TβL β-lactamase	[100]
Bleomycin	*S. verticillus*	Bleomycin acetyltransferase	[222]
Blasticidin S	*Streptoverticillium* strain sp JCM4673	Blasticidin acetyltransferase	[221]
Kasugamycin	*S. kasugaensis*	Kasugamycin acetyltransferase	[81]
Novobiocin	*S. niveus*	Carbamoyltransferase	[107]
Mikamycin B	*S. mitakaensis*	Mikamycin lactonase	[96]
Virginiamycin M_1	*S. virginiae*	Virginiamycin M_1 reductase	[113]
Deoxynivalenol	*F. graminearum*	Acetyltransferase	[97]

S., *Streptomyces*; *B.*, *Bacillus*; *Ps.*, *Pseudomonas*; *F.*, *Fusarium*; *APH* aminoglycoside phosphotransferase, *AAC* aminoglycoside acetyltransferase, *SPH* streptomycin phosphotransferase, *HPH* hygromycin B phosphotransferase, *PAC* puromycin acetyltransferase, *CPT* chloramphenicol phosphotransferase, *VPH* viomycin phosphotransferase, *CPH* capreomycin phosphotransferase, *CAC* capreomycin acetyltransferase, *STAT* streptothricin acetyltransferase, *NAT* nourseothricin acetyltransferase, *MGT* macrolide glycosyltransferase, *(D)PAT* (demethyl)phosphinothricin acetyltransferase, *TβL* tabtoxinine-β-lactam

Hygromycin B

Hygromycin B phosphotransferase (HPH), encoded by *hyg* in *Streptomyces hygroscopicus* [267], acts on the 4-hydroxyl of the hyosamine cyclitol moiety thereby generating inactive 7″-*O*-phosphoryl-hygromycin B [169]. Ribosomes in *S. hygroscopicus* are sensitive to hygromycin B and *hyg* conferred resistance in *S. lividans*, although

whether *S. hygroscopicus* harbours a second candidate resistance gene is unclear. Thus, when two putative HPH-encoding genes were isolated from another producer, *Streptoverticillium eurocidicus*, only one of them hybridized with *hyg*. The second gene found a target elsewhere in *S. hygroscopicus* DNA but the action of its product was not characterized (for additional comments, see [30]). Given that a hypothetical phosphatase is encoded within the hygromycin B gene cluster of *S. hygroscopicus*, it might be that the drug is produced in phosphorylated form and activated during export. But if so, and if there are two HPH activities, at which site(s) is the prodrug phosphorylated?

Streptothricin and Nourseothricin

The streptothricins are potent inhibitors of bacterial ribosomes. Both streptothricin and nourseothricin are inactivated by acetyltransferase activity [73, 92] present in extracts of their producers (*Streptomyces* strain V-13-1 and *Streptomyces noursei*, respectively). Genes encoding such activity (*sta* from *Streptomyces lavendulae* [80], another streptothricin producer; *nat*, formerly *ntr*, from *S. noursei* [105]) confer high-level resistance in *S. lividans* and the product generated by purified streptothricin acetyltransferase (STAT) has been characterized as N^{β}-acetylstreptothricin. The latter compound has antitumour activity but is inert against bacteria [149].

Metabolic shielding

As a radical alternative to target-based resistance, some antibiotic producers protect themselves from endogenous drug by adapting their metabolic lifestyle to defend an intrinsically susceptible target. Besides efficient drug export, a range of options is available to such organisms, including sequestration of intracellular drug molecules or even their (temporary?) inactivation (Table 5). A major strategy involves metabolic shielding, whereby antibiotics are produced and secreted as inert prodrugs that are activated during or following export.

Streptomycin

Ribosomes in *Streptomyces griseus*, and other streptomycin producers, remain fully sensitive to the drug at all times. Streptomycin is produced and secreted as an inactive 6-phosphoryl precursor that is activated during export by a dedicated, extracellular phosphatase, StrK [252], encoded within the streptomycin-biosynthetic (*str-sts*) gene cluster [130]. The exported compound is 6-phosphoryl-dihydrostreptomycin that undergoes 3′-α dehydrogenation prior to activation. During streptomycin production, the aminocyclitol moiety, streptidine, is generated as the 6-*O*-phosphoryl derivative and is maintained in that state in subsequent biosynthetic intermediates to generate the exported species [250]. Free streptomycin can be inactivated by streptomycin 6-phosphotransferase, SPH(6), that is present constitutively in producers [146] and is available to detoxify previously exported drug that might get back into the mycelium. However, as that would set up a futile and costly cycle of ATP consumption, such ingress is presumably limited although not necessarily excluded. As streptidine is also a substrate for SPH(6), this enzyme might help to maintain the inert status of intermediates during biosynthesis, in addition to its role in resistance. The gene encoding SPH(6) is *aphD* that lies within the *str-sts* cluster of *S. griseus* [38] and similar activity is encoded by *sph* in the 5′-hydroxystreptomycin gene cluster of *Streptomyces glaucescens* [248]. Streptomycin is also produced by *Streptomyces bikiniensis*. Metabolic shielding in these various organisms culminates in efflux via a deduced ABC transporter complex, StrVW [10], that is also encoded within the respective biosynthetic gene clusters.

Although not apparent during early studies, two other activities that phosphorylate (dihydro)streptomycin or related compounds are also present in streptomycin producers. A labile SPH(3″) activity [250] encoded by *aphE* (formerly *aphD2*) conferred resistance to streptomycin in *S. lividans* [76]. That gene is not present in the *str-sts* cluster of *S. griseus* (or at all in *S. glaucescens*) and the function of SPH(3″) is unclear. A third activity, 3′-α-phosphotransferase, was originally found in *S. bikiniensis* and utilized 6-phosphoryl-dihydrostreptomycin as substrate [250]. The role(s) of this enzyme is (are) not known and no gene encoding it has been isolated.

Puromycin

Drug-resistant ribosomes are not employed by the puromycin producer *Streptomyces alboniger*. Extracts of this strain contain inducible puromycin *N*-acetyltransferase activity (PAC) that appears late in the growth phase and inactivates the drug [173, 220]. The *pac* gene is present in the puromycin-biosynthetic (*pur*) gene cluster [109] and confers resistance to puromycin when expressed in heterologous hosts [244]. However, during puromycin production, the most likely substrate for PAC is the biosynthetic precursor N^6,N^6,*O*-tridemethylpuromycin-5′-phosphate [226] and thereafter synthesis proceeds via inert *N*-acetylated aminonucleotides. This model therefore differs from earlier biosynthetic schemes that proposed the utilization of non-phosphorylated (aminonucleoside) intermediates. In the final steps before export, the nucleotidic *N*-acetyl-*O*-demethylpuromycin-5′-phosphate is dephosphorylated and then methylated to generate *N*-acetylpuromycin—the

proposed substrate for export by the product of *pur8*. The latter is deduced to encode an efflux protein of the MFS antiporter group. The *pur8* gene also confers specific resistance to puromycin when expressed in *S. lividans* [227]. Following export of *N*-acetylpuromycin, active drug is liberated by *N*-acetylhydrolase activity encoded by *napH* [109] which, like *pur8*, is also present within the puromycin-biosynthetic gene cluster.

Chloramphenicol

Actinomycetes that produce chloramphenicol possess drug-sensitive ribosomes. In non-production medium, *Streptomyces venezuelae* is sensitive to exogenous chloramphenicol that is able to enter the mycelium and block ribosomal function. During the resultant growth lag, the duration of which varies with drug input, chloramphenicol is progressively inactivated. This involves the combined action of a constitutive hydrolase that removes the dichloroacetyl moiety, generating *p*-nitrophenylserinol [154], and chloramphenicol phosphotransferase (CPT) that generates the 3′-*O*-phosphoryl derivative [153]. Both products lack biological activity. In production medium, an additional form of resistance develops concurrently with the appearance of endogenous drug. The producer becomes insensitive to exogenous chloramphenicol and the latter is not detoxified. These observations are consistent with the erection of a drug-influx barrier and/or the establishment of an efflux system [247]. Moreover, the ribosomes must be protected from endogenous drug during idiophase. In clinical isolates and other bacteria, including many *Streptomyces* spp., chloramphenicol resistance is due to 3′-*O*-acetyltransferase (CAT) activity that inactivates the drug. However, despite scrutiny, CAT activity has never been found in chloramphenicol producers, although esterase that could activate 3′-*O*-acetylchloramphenicol was readily detected. For this reason, it was long assumed that chloramphenicol is not synthesized via acetylated precursors. However, 3′-*O*-acetylchloramphenicol has recently been detected in extracts of *S. venezuelae* [66] suggesting that the drug might, after all, be produced in acetylated form and activated by esterase during or after efflux. According to this model, chloramphenicol is simply not a substrate for the enzyme that introduces the acetyl group during biosynthesis.

The cluster of chloramphenicol-biosynthetic genes in *S. venezuelae* [75] does not include a candidate transacetylase or any obvious esterase gene, while *cpt* (encoding 3′-*O*-phosphorylation) and the hydrolase gene are located elsewhere in the genome. However, the cluster does contain candidate efflux genes albeit of unknown significance. The product of *cmlI* is a hypothetical ABC transporter and *cmlF* encodes a deduced MFS exporter similar to chloramphenicol efflux proteins in other organisms [75]. And, outside the cluster, another candidate MFS transporter is encoded by *cmlV* (ORF1 in [153]), a neighbour of *cpt*. A role for *cpt* in self-resistance of *S. venezuelae* has been proposed. Speculatively, CmlV protein might assist by exporting inactive 3′-*O*-phosphorylchloramphenicol [153].

Viomycin and Capreomycin

Cyclic peptides of the tuberactinomycin family inhibit the function of bacterial ribosomes. Capreomycin is produced by *Streptomyces capreolus* (now *Saccharothrix mutabilis* subsp. *capreolus*) as a complex of four components, mainly capreomycins IA and IB with lesser amounts of IIA and IIB. Viomycin is a single molecular species produced by *Streptomyces vinaceus* (now *Streptomyces* sp. strain ATCC 11861). These organisms possess similar phosphotransferase activities (CPH and VPH, respectively) that inactivate capreomycins IA, IIA and viomycin but not capreomycins IB or IIB. Additionally, *S. capreolus* possesses acetyltransferase (CAC) that inactivates all four capreomycins but not viomycin [210]. When genes encoding these activities were cloned in *S. lividans*, *vph* [234] and *cph* [231] conferred high-level resistance to viomycin whereas resistance to capreomycin complex required *cac* [231]. As neither *S. vinaceus* nor *S. capreolus* contained drug-resistant ribosomes in those studies, it seemed plausible that *vph* and *cac* might be crucial resistance determinants in the respective producers. The *vph* gene is present within the viomycin-biosynthetic gene cluster of *S. vinaceus* [232] together with a candidate phosphorylviomycin phosphatase gene [*vioS*; related to *strK*] and also *vioE* that encodes a deduced MFS drug transporter. Perhaps the latter is related to the DNA fragment designated *'vrl'* that conferred low-level viomycin resistance when *vph* was first isolated [234]. In any event, it seems plausible that viomycin might be produced as an inert prodrug (or might be phosphorylated immediately after biosynthesis) and then activated by phosphatase during or after export.

The capreomycin-biosynthetic gene cluster includes *cph* but not *cac* and, when introduced into *S. lividans*, it endowed capreomycin production plus resistance to all four capreomycin components. This observation was taken to demand the presence of another resistance gene within the cluster in addition to *cph* [45]. That gene was proposed to be *cmnU*, deduced to encode an rRNA methylase related to the Kam family that confers resistance to aminoglycosides (as discussed above). As *cmnU* conferred high-level resistance to capreomycin and kanamycin when expressed in *S. lividans* [45] that proposal is plausible, although ribosomes from the capreomycin producer (or from *cmnU*$^+$ strains of *S. lividans*) were not tested for their drug response. Nor was CmnU assayed for rRNA methyltransferase activity that must be inducible in *S. capreolus* as

drug-resistant ribosomes were not observed in earlier studies. Also, *kam* strains generally should be resistant to capreomycin. Within the capreomycin-biosynthetic gene cluster, *cmnE* is deduced to encode an MFS efflux protein, related to VioE. The latter was proposed to transport a phosphorylated compound. It would be interesting to know the state in which the capreomycins are exported.

Oleandomycin

Ribosomes in the oleandomycin producer *Streptomyces antibioticus* are sensitive to the autogenous drug at all times [49]. When this organism was first shown to possess MGT activity that inactivates oleandomycin via 2′-*O*-glucosylation, together with glucosidase that could regenerate active drug, it was proposed that metabolic shielding involving these activities is integral to oleandomycin production [246]. Now it transpires that *S. antibioticus* has two MGT enzymes that display markedly different substrate specificities. OleI, encoded within the oleandomycin-biosynthetic (*ole*) gene cluster, is highly specific for oleandomycin whereas OleD, encoded elsewhere in the genome, modifies a broad range of macrolides with differing lactone ring size and patterns of glycosylation. Accordingly, it was proposed [186] that during antibiotic-biosynthesis OleI acts on the mature drug, and/or its biosynthetic precursors, to prevent the appearance of active drug within the cell. Then, following efflux, 2′-*O*-glucosyl-oleandomycin is activated by an extracellular *β*-glucosidase, OleR [187]. The latter is also encoded within the *ole* cluster along with an ABC transporter, OleB, that exported 2′-*O*-glucosyl-oleandomycin from engineered strains of *S. albus* containing *oleB* and *oleI* [164]. Another ABC transporter, OleC, also conferred resistance to oleandomycin when expressed in *S. albus*. This gene is not part of the *ole* cluster and no specific function for its product has yet been discerned. For example, it is not clear whether OleC can handle 2′-*O*-glucosylmacrolide(s). The extent to which extracellular macrolides in general, including oleandomycin, might gain entry into *S. antibioticus* mycelium is also unclear. Given its broad substrate specificity, OleD seems well suited to protect against such compounds and, purely by way of speculation, OleC might then export them.

(Neo)methymycin

Intriguingly, *Streptomyces venezuelae* ATCC 15439 uses a single polyketide synthase complex (PikAI-PikAV) to generate macrolide products with different lactone ring sizes (methymycin and neomethymycin have 12-member rings; narbomycin and pikromycin have 14-member rings). It is not known whether the larger compounds are made as inert prodrugs, but methymycin and neomethymycin both accumulated extracellularly in 2′-*O*-glucosylated form when *desR* (deduced to encode a secreted *β*-glucosidase) was disrupted within the antibiotic-biosynthetic (*pik-des*) gene cluster of *S. venezuelae* [270]. These glucosylated compounds are inert and DesR might normally activate them during (or after) efflux, although no *mgt* gene or enzymic activity that might contribute to metabolic shielding has been identified in *S. venezuelae*. Nor is it evident whether any putative *mgt* gene would be the primary resistance determinant. The *pik* cluster also contains a geminal pair of deduced *erm*-type genes (*pikR1*, *pikR2*), either or both of which might be expected to confer ribosomal resistance, at whatever level, to the collective antibiotics produced by *S. venezuelae* [261]. However, ribosomes from this organism have not been studied and there the matter rests.

Fosfomycin

This analogue of phosphoenolpyruvate, produced by various *Streptomyces* and *Pseudomonas* spp., inhibits the formation of UDP-*N*-acetylmuramic acid from UDP-*N*-acetylglucosamine at an early stage in peptidoglycan biosynthesis. The fosfomycin producer *Pseudomonas syringae* PB-5123 does not take up exogenous fosfomycin to which it appears totally resistant [61]. Moreover, when a gene (*fosC*) from this organism was cloned in *E. coli*, the resultant strain detoxified fosfomycin and the inert material could be reactivated by alkaline phosphatase. It was therefore proposed that self-resistance in *Ps. syringae* PB-5123 involves phosphorylation of the drug although the authors alluded to the possibility that FosC might also (even preferentially) act on one or more of the fosfomycin-biosynthetic precursors [61]. Concurrently, a DNA fragment from fosfomycin-producing *Streptomyces wedmorensis* was also shown to confer resistance in *E. coli*. Again, the mechanism was drug inactivation with ATP as cosubstrate but here two products, fosfomycin monophosphate and diphosphate, were identified by NMR [108]. That DNA fragment contained two genes (*fomA* and *fomB*) and, when these were cloned separately, resistance was conferred by the former but not by the latter [101]. The product of *fomA* converts fosfomycin to the monophosphate that is subsequently converted to the diphosphate by FomB. But as both derivatives are inactive, what is the point of the second reaction? It was proposed that self-resistance in *S. wedmorensis* is due to FomA. Thereafter, production of the diphosphate would generate an energy-rich molecule, export of which might be coupled to (even driven by) hydrolysis of phosphate ester bonds [101]. The transport system was not identified. However, when fosfomycin-biosynthetic genes from a strain of *Streptomyces fradiae* were analysed [260], revealing close orthologues of *fomA* and *fomB*, three neighbouring genes (*phnDCE*) were

deduced to encode ABC transporter components with great similarity to authentic phosphonate uptake transporters. Plausibly, these could constitute an active phosphonate efflux system.

Bialaphos

Like phaseolotoxin (discussed above) and tabtoxin (see below), bialaphos is a Trojan Horse from which a highly toxic 'warhead' is liberated via non-specific proteolysis. Bialaphos is a tripeptide (phosphinothricyl-alanyl-alanine) produced by *Streptomyces hygroscopicus* ATCC 21705 and *Streptomyces viridochromogenes* Tü494. The toxic moiety, phosphinothricin, is an analog of L-glutamate that inhibits glutamine synthase, including the enzymes from bialaphos producers, whereas the tripeptide is inert in vitro. Plants are especially sensitive to the tripeptide and to phosphinothricin (both of which they take up) because glutamine synthase is needed for detoxification of ammonia and pH maintenance in chloroplasts. Although phosphinothricin gets into plants, it does not penetrate into bacteria. However, bacterial oligopeptide transport systems recognise the tripeptide, which is taken up and then cleaved with toxic consequences. In bialaphos producers, phosphinothricin is inactivated by acetyltransferase activity (phosphinothricin *N*-acetyltransferase, PAT) that appears at the onset of toxin production [84] and genes encoding such activity (*bar* from *S. hygroscopicus* and *pat* from *S. viridochromogenes*) confer resistance to bialaphos when cloned in *S. lividans*, even though the tripeptide is not a substrate for PAT [219, 233]. Likewise in producers, uptake of bialaphos is followed by activation and detoxification, although the tripeptide can be toxic if added at early stages of growth prior to the appearance of PAT. However, phosphinothricin is not a biosynthetic precursor of bialaphos and the substrate for acetylation during toxin production is the toxic compound demethylphosphinothricin. Biosynthesis then continues via a benign route to the tripeptide *N*-acetyldemethylbialaphos, within which the demethylphosphinothricin moiety is doubly detoxified. Therefore, following methylation, the tripeptide can be deacetylated without activation to generate bialaphos [233]. Potential for usage of bialaphos and phosphinothricin as selective weedkillers was demonstrated when transgenic plants (such as tomato, tobacco or potato) containing *bar* [36] or *pat* [259] proved to be totally resistant.

Tabtoxin

This phytotoxin, produced by *Pseudomonas syringae* pv. *tabaci*, causes wildfire disease in tobacco. Tabtoxin is a dipeptide containing tabtoxinine-β-lactam linked to threonine (or serine) from which proteolysis releases tabtoxinine-β-lactam that irreversibly inactivates glutamine synthase. Although adenylylation of that enzyme in the producer reduced its sensitivity to tabtoxinine-β-lactam, self-protection was attributed primarily to β-lactamase activity [100]. Thus, the ring-opened, inactive species, tabtoxinine, accumulated when *Ps. syringae* pv. *tabaci* was fed with tabtoxinine-β-lactam, which was evidently not excluded from the producer. In this same organism, the gene *ttr* encodes a second enzyme capable of inactivating tabtoxinine-β-lactam. The TTR protein also opens the β-lactam ring, in this case via acetylation on the imide nitrogen [74], and *ttr* confers resistance to tabtoxin in *E. coli* and in transgenic tobacco plants [3]. However, a role in resistance might not represent the principal function of TTR. Tabtoxin biosynthesis proceeds via a pathway of inert intermediates, as revealed by accumulation of an *N*-acetylated tabtoxinine precursor in mutants of *Ps. syringae* pv. *tabaci* [44]. That *N*-acetyl group (perhaps introduced by TTR) prevents formation of the β-lactam ring that is essential for toxicity. Thus, delay of deacetylation until after dipeptide formation ensures that the biosynthetic precursors of tabtoxin (and the toxin itself, prior to proteolysis) are all inert. TTR might also recycle tabtoxinine, generated via the β-lactamase, back into the biosynthetic pathway.

Resistance was restored in deleted mutants of *Ps. syringae* pv. *tabaci* by a block of three genes from the tabtoxin-biosynthetic cluster [98, 99], one of which (*tblR*) is deduced to encode an MFS drug transporter. Also present in the cluster is *tabP* that encodes a hypothetical zinc metalloprotease, perhaps responsible for tabtoxin activation, but there is no candidate β-lactamase gene in the cluster and, curiously, *ttr* is also absent.

β-Lactams

Whether or not they produce β-lactams, most *Streptomyces* spp. are quite resistant to benzylpenicillin. Constitutive production of extracellular β-lactamase is the norm for these organisms but there is no obvious correlation between levels of β-lactam resistance and the presence of β-lactamases (or even the levels of their production). Also, many of the penicillin-binding proteins (PBPs) encountered among *Streptomyces* spp. display comparatively low affinities for β-lactams [157]. Hence, although resistance in the producers of β-lactams most likely involves interplay between β-lactamases and, especially, low-affinity PBPs, the significant details may not always be obvious. Nevertheless, in the cephamycin C-biosynthetic gene cluster of *Amycolatopsis* (formerly *Nocardia*) *lactamdurans*, *pbp40* encodes a low-M_r PBP related to D, D-carboxypeptidases while *bla* encodes an extracellular β-lactamase and both have proposed roles in resistance [120]. Thus, in engineered strains,

overexpression of *bla* raised the MIC for benzylpenicillin whereas disruption of that gene had the opposite effect.

More intriguingly, *Streptomyces clavuligerus* produces two β-lactam antibiotics, cephamycin C and clavulanic acid. Genes encoding biosynthesis of these compounds are arranged in a 'supercluster' as two adjacent blocks each of which includes a β-lactamase (*bla*) gene [86, 120] and, significantly, the *bla* gene within the clavulanic acid cluster is essential for production of that compound [116]. Also present in this cluster are two *pbp* genes [140] although their function is unclear as neither is essential for clavulanic acid production [87]. Within the cephamycin C cluster, *pcbR* encodes a membrane-associated protein with a low affinity for benzylpenicillin and a clear role (from gene disruption analysis) in resistance to cephalosporins [168], while *pbp74* encodes another high-M_r PBP [174]. In addition to producing two β-lactams, *S. clavuligerus* also produces 'β-lactamase inhibitory protein' (BLIP; [40]). This protein has been purified, its complex with β-lactamase has been studied in detail, and its action is presumably significant in an organism that produces at least two β-lactamases. However, BLIP is not encoded within the *S. clavuligerus* supercluster although the cephamycin gene block does include *blp* that encodes a hypothetical protein related to BLIP. Indeed, *S. clavuligerus* appears well endowed with β-lactamase inhibitors, given that clavulanic acid is widely used as such in human medicine. Regarding drug export, a deduced MFS efflux gene (*cmcT*) present in the cephamycin C cluster of *S. clavuligerus* has an orthologue in *A. lactamdurans* [120] while another candidate efflux gene (*orf13*) resides in the clavulanic acid cluster [140]. Currently, there is no overarching model for presumed interplay between these various components in determining production of the antibiotics native to *S. clavuligerus* and the well-being of the producer. But already there is the basis of a compelling story.

Resistance involving drug-binding proteins

Lantibiotics, Microcins, Thiopeptides (Thiocillins)

A remarkable range of antibiotics, variously classified as lantibiotics, microcins or thiopeptides (thiazolylpeptides or thiocillins), share a common biosynthetic strategy and, in that respect, are more closely related than was formerly appreciated. These compounds are not, after all, produced on giant assembly-line enzymes of the non-ribosomal peptide synthase (NRPS) family. Despite their small size, they are synthesized on ribosomes as precursor peptides that are later subjected to extravagant levels of post-translational modification prior to activation by cleavage [114, 196, 257]. Typically, the precursor peptide might be 50–70 residues long (69 for microcin B17; 58 for thiostrepton; 57 for the lantibiotic, nisin) giving rise to a structural peptide (active drug) derived from a much shorter segment (43 residues for microcin B17; 17 for thiostrepton; 34 for nisin) after removal of a leader peptide sequence.

Lantibiotics

These drugs target the bacterial membrane where they variously form pores and/or block cell wall biosynthesis by attachment to lipid II, the membrane-bound disaccharidic-oligopeptide intermediate in peptidogycan biosynthesis [16]. Lantibiotic-biosynthetic gene clusters (some of which are plasmid-borne) typically include '*lanA*' that encodes the precursor peptide, '*lanP*' that specifies a protease for activation, various genes for post-translational modification, and '*lanT*'. The last of these usually encodes an ABC transporter polypeptide, complete with ATP-binding and membrane-spanning domains, that functions as a homodimer and exports the lantibiotic from the producing cell. In the case of NisT, that means exporting the nisin precursor peptide after post-translational modification but before cleavage. (Some lantibiotics are cleaved before export.) Immunity systems in the respective producers are also encoded within the biosynthetic gene clusters and typically consist of 'LanI', a membrane-associated protein (or peptide; some are quite short) plus 'LanFEG', an ABC transporter with multiple components. Characteristically, the LanFEG transporters are much more specific for the mature drug than are the LanT systems [139]. When genes from the nisin producer *Lactococcus lactis* were expressed in *Bacillus subtilis*, both *nisI* and *nisFEG* separately conferred significant levels of nisin resistance although both were needed for optimal protection [218]. Nisin is exported prior to activation and its sole target, lipid II, is accessible from outside the cell. The NisI immunity protein has an *N*-terminal signal sequence plus a membrane anchor and attaches to the outer surface of the cytoplasmic membrane. From there, NisI can intercept and sequester its cognate lantibiotic, thereby giving protection against external drug [218]. Other LanI proteins are proposed to act in similar fashion. Being a transmembrane protein complex, the immunity ABC transporter can contribute to resistance by removing embedded lantibiotic from the membrane and ejecting it. Thus, lantibiotic resistance principally features highly specific drug-binding proteins.

Microcins and Thiopeptides (Thiocillins)

Bacterial microcins (not to be confused with bacteriocins, which are much larger) and thiopeptides related to thiostrepton differ from lantibiotics in attacking intracellular targets. Thus, microcin B17 (MccB17) inhibits DNA

gyrase; MccJ25 targets RNA polymerase; thiopeptides (thiocillins) bind to ribosomes. However, in a manner reminiscent of lantibiotic immunity, a broad range of microcin-producing bacteria utilize cooperative activities of soluble immunity proteins plus ABC transporters to achieve resistance via drug sequestration and efflux [121]. In that context, recent work with a strain of *Bacillus cereus* that produces micrococcin P (variously referred to as a thiopeptide or a thiocillin) has given drug sequestration a potentially fascinating twist. In addition to the expected ABC transporter, the gene cluster that encodes micrococcin biosynthesis includes two orfs (*tclQ* and *tclT*) that are each deduced to encode ribosomal protein L11 [257]. Can this be coincidental? When micrococcin binds to ribosomes, it interacts in cooperative fashion with protein L11 and 23S rRNA [184] and resistance can be achieved via mutations specifically affecting that protein [215]. Does *tclQ* and/or *tclT* encode a functional version of protein L11 that might be incorporated into ribosomes to mediate resistance? Although ribosomes from the original micrococcin producers were not resistant to the drug [39], it is possible that *tclQ* and/or *tclT* might be expressed only under specific conditions that did not pertain in those earlier studies. Alternatively, might the TclQ and/or TclT protein(s) act as decoy binding sites for the drug? And, in any event, why two?

Bleomycin

In organisms that produce DNA-binding drugs, self-protection strategies rely heavily on drug sequestration associated with efficient efflux systems.

Bleomycin, phleomycin and related glycopeptides (including tallysomycin) introduce single-strand breaks into DNA in the presence of molecular oxygen and metallic ions such as Fe(II). The bleomycin producer *Streptomyces verticillus* contains bleomycin acetyltransferase activity (BAT; the product of *blmB*) that inactivates bleomycin, but not phleomycin. Also present is an acidic protein, encoded by *blmA*, that sequesters bleomycin and thereby prevents DNA damage [222]. A similar bleomycin-binding resistance protein (BRP, the product of Sh *ble*) had earlier been found in the tallysomycin producer *Streptoalloteichus hindustanus*, and protease treatment of the drug–protein complex had restored DNA-damaging activity, indicating that the drug was unaltered by the protein [62]. When *blmA* and *blmB* were expressed separately in *S. lividans* or *E. coli* they each conferred resistance to bleomycin [222], but it is not known whether these genes might cooperate in determining self-resistance in bleomycin producers. Nor is it clear whether BlmA or BlmB might interact directly with a drug-efflux mechanism [23] or whether bleomycin is exported in mature or acetylated form.

Mitomycin C

This antitumour agent binds into the minor groove of DNA, causing monofunctional alkylation and/or lethal intra- or interstrand cross-linking. Alkylation occurs at 5′ CpG 3′ sequences that abound in the high-GC DNA of actinobacteria, such as the mitomycin C producer *Streptomyces lavendulae*. Accordingly, self-protection in this organism requires that active drug molecules be kept away from a large number of potentially susceptible target sites. The products of at least three genes are involved in achieving this goal. Mitomycin C is synthesized as an inert prodrug that is activated within *S. lavendulae* in a two-step process, involving reduction via catalytic electron transfer followed by molecular rearrangement to generate the alkylating agent (quinone methide). The first step is reversible by McrA, an inducible flavoprotein that reoxidizes reduced mitomycin C [6]. Also in the producer, native prodrug can be sequestered by a soluble protein, Mrd, and delivered to Mct for efflux [204]. The latter is deduced to be an integral membrane protein of the MFS antiporter class [205]. In support of this model, each of the respective genes (*mcrA*, *mrd*, *mct*) conferred resistance to mitomycin C when expressed separately in *S. lividans* or *E. coli* whereas disruption of *mct* in the *S. lavendulae* genome resulted in hypersensitivity. When *mrd* was expressed together with *mct* in *E. coli*, intracellular accumulation of radiolabelled mitomycin C was significantly reduced and high levels of resistance were achieved [205]. Such cooperative function of Mrd with Mct generates resistance via drug sequestration allied to efflux. These two proteins are encoded within the mitomycin C-biosynthetic gene cluster whereas McrA is not.

Daunorubicin-Doxorubicin

Anthracyclines, such as daunomycin (daunorubicin) and the related doxorubicin (adriamycin), bind to DNA via sequence-specific intercalation. Once bound, the drug can be enzymically reduced to an anthraquinone that reacts with molecular oxygen in the presence of metal ions to generate hydroxyl radicals [43], which cause DNA damage including strand breakage. In the absence of DNA damage, the bound drug inhibits transcription and DNA replication.

Three genes (*drrA*, *drrB*, *drrC*) from the daunorubicin-biosynthetic cluster of *Streptomyces peucetius* confer daunorubicin-doxorubicin resistance when expressed in *S. lividans* or *E. coli*. In *S. peucetius*, these genes are expressed only during idiophase, with *drrC* being transcribed earlier than *drrA* and *drrB*. The DrrA and DrrB proteins act together as a drug-efflux complex, deduced to be of the ABC family [67, 90]. In contrast, DrrC resembles UvrA of *E. coli* and binds to DNA although, unlike UvrA,

it does not repair DNA damage [125]. Binding of the purified protein to DNA is dependent on ATP and is strongly stimulated by daunorubicin, leading to speculation that DrrC displaces daunorubicin from DNA intercalation sites and/or inhibits DNA damage by preventing reduction of bound drug [57]. All three genes are inducible by daunorubicin. Synthesis of DrrC begins when daunorubicin first appears and *S. peucetius* DNA is thereby protected until the DrrAB transporter establishes drug efflux.

Chromomycin A_3

The chromomycin A_3 producer *Streptomyces griseus* subsp. *griseus* is highly resistant to its product but sensitive to the closely related compound mithramycin. The converse holds for the mithramycin producer *Streptomyces argillaceus*. When three candidate resistance determinants (*cmrA*, *cmrB*, *cmrX*) from the chromomycin A_3-biosynthetic gene cluster [142] were cloned in *Streptomyces albus*, all three genes were required for high-level resistance [141]. The combination of *cmrA* plus *cmrB* was much less effective while *cmrX* alone gave only low-level resistance. When *cmrX* was disrupted in *S. griseus*, chromomycin A_3 production was reduced and the strain became less resistant, whereas disruption of *cmrAB* was not possible in a production strain. The deduced product of *cmrX* resembles UvrA of *E. coli* and DrrC of *S. peucetius*, leading to speculation that it too might bind to DNA and thereby prevent binding of chromomycin A_3 to DNA and/or cause release of pre-bound drug [141]. The combined products of *cmrA* and *cmrB* form a hypothetical ABC drug transporter that was initially expected to drive efflux of chromomycin A_3 from the producer [142]. However, the penultimate (di-deacetylated) compound, DDACA3, in the chromomycin A_3-biosynthetic pathway proved to be the preferred substrate for export [141]. It was therefore proposed that self-resistance of *S. griseus* has two aspects: CmrX protects against the low-level activity of biosynthetic precursors of chromomycin A_3 (including DDACA3) while export of DDACA3 by CmrAB is coupled to diacetylation by the transmembrane protein CmmA [143], to generate highly toxic chromomycin A_3 outside the cell.

Mithramycin

In *S. argillaceus*, self-resistance to mithramycin depends primarily on a deduced ABC transporter encoded by *mtrA* and *mtrB* [47], genes that are closely related to *cmrA* and *cmrB* of the chromomycin A_3 producer. An adjacent gene, *mtrX* (similar to *cmrX*) was not required for mithramycin resistance in engineered *S. albus* strains harbouring *mtrA plus mtrB*, but was not tested for possible ability to supplement the effects of *mtrAB*.

Enediynes

There are two types of enediyne antibiotics. The 'chromoproteins' (including neocarzinostatin, maduropeptin and C-1027) consist of a 9-member enediyne core attached to an acidic apoprotein. The latter is added to the core as a pre-apoprotein prior to removal of a leader peptide sequence. The apoprotein stabilizes the enediyne 'warhead' and aids binding to DNA. Thereafter, reductive activation (e.g. by thiols) triggers formation of a 1,4-dehydrobenzene diradical that causes double strand scission of DNA ('chemical nuclease' activity). Other enediynes, including calicheamicin and dynemicin, possess a 10-member core, lack apoproteins, and fall into two subgroups: either an oligosaccharidic 'docking' module (as in calicheamicin) or an anthracycline (in dynemicin) selects the binding site in the minor groove of DNA prior to reductive activation and DNA breakage [239]. All enediynes are active against DNA from whatever source and the actinomycetes that produce them face a daunting challenge.

Various possibilities can be envisaged for self-resistance to enediynes [58]. Producers of the chromoproteins depend on the apoprotein moiety for their survival and there is also heavy reliance on efficient drug export from the various producers. Thus, gene clusters for biosynthesis of maduropeptin (in *Actinomadura madurea*, [242]); neocarzinostatin (*Streptomyces neocarzinostaticus*, [124]); C-1027 (*Streptomyces globisporus*, [123]); dynemicin (*Micromonospora chersina*, [60]) and calicheamicin (*Micromonospora echinospora* spp. *calichensis*, [2]) each encode a deduced MFS efflux antiporter. Additionally, the calicheamicin cluster possesses a putative ABC transporter, while there are several such candidates in the dynemicin cluster. Genes hypothetically associated with DNA damage repair (or prevention) are also located in the madurapeptin, C-1027 and dynemicin biosynthetic clusters. For example, SgcB2 encoded within the C-1027 gene cluster is similar to DrrC of *S. peucetius* and might destabilize binding of C-1027 to DNA. But, most famously, the calicheamicin producer uses the CalC protein as a decoy drug target [12]. The warhead of calicheamicin cleaves CalC by abstracting hydrogen from residue G113, resulting in quenched, inactive calicheamicin ε and fragmented CalC (residues 1–113 and 114–181). Such 'self-sacrifice' constitutes a unique resistance mechanism.

Contribution of impaired drug entry to resistance

There is much anecdotal evidence that influx of antibiotics into the organisms that produce them might be impaired and this notion has its attractions, not least because futile cycles of active efflux and drug re-importation make little sense. For

example, as discussed elsewhere in this article, *Ps. syringae* PB-5123 was reported not to take up its product, fosfomycin [61]. Various macrolide producers, some of which routinely display inducible resistance to their products, also display selective resistance when uninduced and in non-production media. For example, while their ribosomes remained drug-sensitive, and in the absence of drug inactivation, the producers of tylosin (*S. fradiae*), spiramycin (*S. ambofaciens*) or carbomycin (*Streptomyces tendae, Streptomyces halstedii*) did not take up their respective products from growth media and were selectively resistant to them [50]. With other organisms, accumulation of drug from the medium was reduced during idiophase, as when *Streptomyces refuineus* became insensitive to its product, anthramycin, but not to closely related compounds [192]. In a somewhat similar scenario, the chloramphenicol producer *S. venezuelae* is sensitive to exogenous chloramphenicol when grown in non-production media, under which conditions ribosomal function is blocked while the drug is progressively detoxified. But during idiophase, exogenous chloramphenicol causes no growth lag although the ribosomes remain sensitive, and the drug is not inactivated even though the responsible enzymes are still produced [247]. Of course, such observations, no matter how suggestive, cannot be interpreted unambiguously unless reduced drug influx can be distinguished from enhanced efflux.

But none of this dispels the abiding impression that self-protection of antibiotic-producing organisms might commonly involve impaired (re)entry of toxic products.

Resistance via drug efflux

Perhaps inevitably, drug-export systems are fundamentally involved in resistance to membrane-active antibiotics that themselves mediate transmembrane ion movements. So it is with the polyene and polyether ionophores.

Polyenes

The gene clusters that encode biosynthesis of candicidin [24], amphotericin [20], nystatin [15] or pimaricin [4] each contain a pair of genes deduced to encode an ABC drug transporter. Three of these putative transporters are closely similar whereas that for candicidin more closely resembles DrrAB from the daunorubicin producer. The pimaricin gene cluster also harbours *pimH* that encodes a hypothetical MFS antiporter.

Polyethers

Deduced MFS drug transporters are encoded in the biosynthetic gene clusters for monensin (MonT; [165]) and nanchangmycin (dianemicin; NanT1; [223]) in *Streptomyces cinnamonensis* and *Streptomyces nanchangensis*, respectively. In contrast, DNA from *Streptomyces longisporoflavus* that conferred resistance to tetranasin when cloned in *S. lividans* or *S. albus*, was deduced to encode two complementary components of an ABC drug exporter, TnrB [119].

Tetranactin

A gene, *nonR*, from *Streptomyces griseus*, producer of the macrotetralide tetranactin, conferred resistance when cloned in *S. lividans* and was deduced to encode a serine protease or esterase [180]. Although the latter might well inactivate tetranactin, it is not clear how that would allow for production of active drug. No other candidate resistance gene is present in the tetranactin-biosynthetic gene cluster [249].

Bacillus peptides

Genes deduced to encode ABC transporters are present in the clusters that encode biosynthesis of various cationic peptides in bacilli. These include *pmxCD* in the polymyxin producer *Paenibacillus* (formerly *Bacillus*) *polymyxa* [26]; *tycDE* in *Bacillus brevis*, producer of tyrocidine [152]; and *bcrABC* in the bacitracin producer *Bacillus licheniformis* [181]. A role for *bcrABC* in conferring resistance to bacitracin has been established. Less obvious determinants of resistance might also be associated with production of polymyxin-related peptides. For example, colistin binds to lipopolysaccharides and acidic phospholipids within cytoplasmic membranes that, in colistin producers such as *Bacillus colistinus*, contain much less phospholipid compared with membranes of susceptible bacteria [206].

Tetracenomycin and other anthracyclines

Resistance to tetracenomycin C in *S. glaucescens* is inducible by tetracenomycin C and conferred by *tcmA*. The latter gene is negatively controlled by the product of its neighbour (*tcmR*) in much the same way that TetR regulates *tetA*. Thus, TcmR binds to DNA between the divergent *tcmA*-*tcmR* genes and tetracenomycin C disrupts such binding [68]. The TcmA protein is deduced to be an MFS antiporter and *tcmA* is the only candidate resistance gene yet found in *S. glaucescens* [69]. Similarly, within other anthracycline-biosynthetic gene clusters, deduced MLS antiporters are encoded by *elmE* in *Streptomyces olivaceus*, producer of elloramycin [188] and by *sfrB* in "*Streptomyces steffisburgensis*", the producer of steffimycin [70]. In contrast, DrrAB in the daunomycin producer is an ABC transporter.

Simocyclinone D8

This novel antibiotic possesses an aminocoumarin moiety but inhibits DNA gyrase by binding to the A subunit [52] and, unlike the producers of aminocoumarins (such as novobiocin) that target the gyrase B subunit, *Streptomyces antibioticus* does not employ a drug-resistant gyrase in order to achieve self-resistance to simocyclinone [110]. A gene from *S. antibioticus* that conferred simocyclinone resistance in *S. lividans*, did so only when expressed at abnormally high levels using a foreign promoter [11]. This gene, previously referred to as *simEX1* [241] or *sim17* [59], was designated *simX* in the later analysis [110] and its product was deduced to be an MFS drug antiporter. As the level of resistance in *S. lividans* was only marginal when *simX* was expressed from its native promoter, the authors concluded that no definitive role for this gene in self-resistance of *S. antibioticus* could be inferred. Other genes referred to in this article have been touted as bona fide resistance determinants on less evidence.

Expression of *simX* is controlled by the upstream divergent gene *simR*, in a manner reminiscent of *tetA* control by *tetR* (as discussed above). Interestingly, *simX* was induced (i.e. the repressor SimR was displaced from *simR*-*simX* intergenic DNA) not only by simocyclinone D8 but also by simocyclinone C4, a biosynthetic precursor that lacks gyrase-inhibitory activity. Hence, no matter what the significance of its contribution to drug efflux or resistance might be, SimX is likely to be present at early times during simocyclinone production. This type of regulatory device is probably widespread and recently acquired the evocative sobriquet 'feedforward activation' in the context of induced expression of the actinorhodin efflux genes (*actII-orf2* and/or *actVA-orf1*) by precursors of the drug in *S. coelicolor* [224]. And, of course, to complement feedforward, drug precursors can also exert 'feedback activation' in their regulation of biosynthetic events. For example, during tylosin production, synthesis of the polyketide aglycone is massively stimulated by trace amounts of its glycosylated derivatives [51]. In this way, expensive polyketide metabolism is presciently minimized pending the availability of deoxyhexose(s) for ongoing metabolism.

Actinorhodin

Originally, transplantation of the entire *act* gene cluster had rendered *Streptomyces parvulus* capable of actinorhodin production minus suicide [128] and sequence similarities with *tetA* suggested later that *actII-orf2* [48] and *actVA-orf1* [19] were resistance proteins involved in actinorhodin export. In a recent report alluded to above [224], *actII-orf2* and the divergent *actII-orf1* were conveniently designated '*actA*' and '*actR*' to emphasize similarities with the *tet* archetype. The status of blue pigments in *S. coelicolor* has been problematic as not all such material is actinorhodin. Gene disruption analysis has indicated roles for both ActII-orf2 and ActVA-orf1 in export of blue pigments although the suggested 'cooperativity' between these proteins (both are deduced MFS transporters) in protecting *S. coelicolor* against actinorhodin [19] could perhaps be re-phrased as 'complementarity'.

Streptogramins

Streptogramins are produced as a synergistic complex of two components: a polyunsaturated peptide–polyketide macrolactone (A component) plus a cyclic hexa (or hepta) depsipeptide (B component). Among the better-known streptogramins (A and B components, respectively) are virginiamycins (M and S), and pristinamycins (IIA and IA). Although *erm* genes are widely distributed among Gram-positive bacteria and confer resistance to streptogramin B antibiotics via rRNA methylation, such resistance is curiously not encountered in streptogramin producers. Rather, ring opening (mikamycin B hydrolase) has been reported in *Streptomyces mitakaensis* [96] as has inactivation of virginiamycin M_1 (via stereospecific reduction of the C-16 carbonyl) in *Streptomyces virginiae* [113]. Otherwise, reports of resistance in streptogramin producers have exclusively involved efflux proteins. Thus, in *S. virginiae*, a supercluster of genes for synthesis of virginiamycins M and S encodes: VarS, a deduced MFS antiporter for virginiamycin S [112]; an ABC transporter (VarM) specific for virginiamycin M; and another ABC transporter (VarL) of uncharacterised specificity [185]. When cloned in *S. lividans*, the *ptr* gene from *Streptomyces pristinaespiralis* gene (deduced to encode an MFS transporter) conferred resistance to the IA and IIA components of pristinamycin (separately or in combination) and to the unrelated antibiotic rifamycin [14]. The promoter of this remarkable gene exhibits a metabolic 'stress response', being induced not only by the pristinamycins but also by other antibiotics (unrelated to pristinamycin by structure or mode of action) to which *ptr* does not even confer resistance [197].

Concluding remarks

Antibiotic-resistance determinants were already widely distributed in the environment prior to onset of the 'antibiotic era' (as defined by industrial production and clinical usage) and multiple resistance was endemic among organisms unlikely to have been affected by human dispersal of antibiotics. This was emphasized following a recent survey in which soil-derived actinomycetes were challenged with a battery of 21 antibiotics [35]. The latter

was a fair representation of drugs that are commonly used in human medicine and included synthetic antimicrobials alongside natural products and their semisynthetic derivatives. The organisms screened in this study presumably included many antibiotic producers although this point was not assessed specifically. Hence, it is not clear to what extent unrecognised producers might have contributed to the "previously under-appreciated density and concentration of environmental antibiotic resistance" [35], apparently involving novel mechanisms, that was unearthed (sic). Antibiotic producers have long been indicted as likely sources of resistance determinants acquired by clinical isolates [9, 251] and an untapped reservoir of such donor strains presumably still remains. Given current problems with clinical antibiotic resistance, our present topic has never been more pertinent than now. The literature search for this article was concluded in June 2009.

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