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Antibiotics: Opportunities for Genetic Manipulation

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Antibiotics: opportunities for genetic manipulation

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New antibiotics can still be discovered by the development of novel screening procedures. Notable successes over the last few years include the monobactams, β-lactamase inhibitors (clavulanic acid) and new glycopeptides in the antibacterial field; antiparasitic agents such as avermectins; and herbicidal antibiotics like bialaphos. In the future we can expect the engineering of genes from 'difficult' pathogens, including mycobacteria and fungi, and cancer cells, to provide increasingly useful *in vitro* targets for the screening of antibiotics that can kill pathogens and tumours. There will also be a greater awareness of the need to reveal the full potential for antibiotic production on the part of microorganisms by the physiologial and/or genetic awakening of 'silent' genes. Nevertheless, the supply of natural antibiotics for direct use or chemical modification is not infinite and there will be increasing scope for widening the range of available antibiotics by genetic engineering. 'Hybrid' antibiotics have been shown to be generated by the transfer of genes on suitable vectors between strains producing chemically related compounds. More exciting is the possibility of generating novelty by the genetic engineering of the synthases that determine the basic structure of antibiotics belonging to such classes as the β-lactams and polyketides. Research in this area will certainly yield knowledge of considerable scientific interest and probably also of potential applicability.

In the improvement of antibiotic titre in actinomycetes, protoplast fusion between divergent selection lines has taken a place alongside random mutation and screening. In some cases the cloning of genes controlling metabolic 'bottlenecks' in fungi and actinomycetes will give an immediate benefit in the conversion of accumulated biosynthetic intermediates to the desired end product. However, the main impact of genetic engineering in titre improvement will probably come only after a further use of this technology to understand and manipulate the regulation of antibiotic biosynthesis as a facet of the general challenge of understanding differential gene expression. *Streptomyces* offers a particularly fertile field for such research, following the isolation of DNA segments that carry groups of closely linked operons for the biosynthesis of and resistance to particular antibiotics, and of genes with pleiotropic effects on morphological differentiation and secondary metabolite formation.

1. Introduction

The starting point for this paper is an article called 'Fresh approaches to antibiotic production' (Hopwood & Chater 1980) written for a previous Discussion Meeting of the Royal Society held under the title 'New Horizons in Industrial Microbiology' in May 1979, just before publication of the Spinks Report. Re-reading that article, I am struck by the fact that *in vitro* techniques for genetic manipulation of antibiotic-producing microorganisms and their use to isolate and study antibiotic biosynthetic genes are all post-Spinks. The first reports of gene cloning in *Streptomyces* appeared in 1980 (Bibb *et al.* 1980; Suarez & Chater 1980; Thompson *et al.* 1980) and genes controlling steps in antibiotic biosynthesis were first isolated in 1983 (Feitelson & Hopwood 1983; Gil & Hopwood 1983; Chater & Bruton 1983). For filamentous fungi, the corresponding dates were 1981 (Schweizer *et al.* 1981) and 1985 (Samson *et al.* 1985).

Because the resulting advances in genetic knowledge and possibilities for manipulation represent a significant qualitative change in the science pertaining to antibiotics in the last eight years, I devote much of this article to them.

Whatever the possibilities for using genetic approaches to antibiotic production turn out to be, it will take some time for applications to emerge; thus the situation is no different from the use of genetic engineering to discover or produce useful materials in other areas of biology or from the development of antibiotics by more traditional methods. Moreover, genetic manipulation will clearly not replace, but only complement, the production of new antibiotics by the screening of natural isolates, and the improvement of antibiotic titre by mutation combined with medium and fermentation optimization. This article therefore tries to put the genetic approaches in perspective by considering what has happened over the last eight years in these more familiar areas of antibiotic discovery and development.

2. Production of new antibiotics

(*a*) *The continuing need for new compounds*

Eight years ago we grouped the needs for new antibiotics under four headings: new antibacterial agents; better anti-fungal, anti-tumour and anti-viral antibiotics; growth promotion without cross resistance; and control of plant pathogens and invertebrates (Hopwood & Chater 1980). These headings are still appropriate, although there has been some change in the relative emphasis in different areas.

The antibacterial field has seen a growth in the importance of infections caused by multiply antibiotic-resistant Gram-positive bacteria, leading to increased interest in discovering novel antibiotics of the glycopeptide class (to which the already valuable vancomycin belongs), such as the aridicins (Sitrin *et al.* 1985) and teicoplanin (Parenti *et al.* 1977). The need for new agents to treat diseases caused by aerobic and anaerobic Gram-positive bacteria also prompted development of the novel lipopeptide antibiotic LY146032 (Huber *et al.* 1986). The continuing importance of tuberculosis and leprosy in many parts of the world, and the increased incidence of infections caused by mycobacteria of the *Mycobacterium avium–intracellulare–scrofulaceum* complex in AIDS patients (Mitchison *et al.* 1988), has stimulated interest in the search for more effective anti-mycobacterial drugs, but it is too early to judge the potential of these efforts.

In the anti-fungal, anti-tumour and anti-viral fields, where the achievement of selective killing of the parasite or cancer cell is such a major problem, fundamental progress in the discovery of new chemotherapeutic agents has been limited. It is not clear why it has proved so difficult to target the special biochemical properties of the fungal outer envelope more effectively, but one problem is that the medically important fungi are difficult to adapt to efficient screens. For the treatment of tumours and viruses, there is probably more current interest in modifiers of the immune system than in traditional chemotherapeutic agents, although the realization that the reverse transcriptase of retroviruses, such as that causing AIDS, may represent a chink in their armour has stimulated the search for compounds that inhibit it (Sarin 1988).

Agriculture represents a very important area for the development of new antibiotics because of the range of their possible applications. Over the last eight years, growth-promoting antibiotics quite distinct from those used clinically have continued to be a strong interest. Added to this have been such advances as the introduction of an avermectin derivative (ivermectin: Campbell *et al.* 1983) to treat invertebrate infestations of farm animals (with a

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significant hope that it will also be useful in the control of parasitic diseases such as river blindness in man); and of bialaphos, the first natural product herbicide (Tachibana 1987), with the added advantage of a resistance gene in the producing organism that can be used to confer selective resistance when cloned into crop plants (De Block *et al.* 1987). The search for antibiotics to control plant pathogens is likely to increase significantly in the future; interest in this topic in China is noteworthy.

Small-molecular-mass enzyme inhibitors, immunomodifiers and other pharmacologically active agents of microbial origin (Umezawa 1988) are logically grouped with antibiotics. Cyclosporin is already in use as an immunomodifier and mevinolin as an anti-hypercholesterolaemic agent. There is a significant need for, and interest in, the discovery of further novel metabolites of these types (Badger 1983; Nisbet & Westley 1986).

(*b*) *Chemical modifications of known antibiotics*

Although certain antibiotics, such as oxytetracycline or erythromycin A, are apparently ideally suited for application in their naturally produced form, chemical modifications will usually improve the properties of the compound, as was dramatically demonstrated in the classical case of the semi-synthetic penicillins and cephalosporins. A more recent example is ivermectin, derived from the natural mixture of avermectins by hydrogenation (Campbell *et al.* 1983). The classical technique of directed biosynthesis, used to produce penicillin V by feeding the *Penicillium* fermentation with the artificial side-chain precursor phenoxyacetic acid (Behrens *et al.* 1948), has found recent use in the production of LY146032 by controlled feeding of a *Streptomyces roseosporus* fermentation with decanoic acid (Huber *et al.* 1986). In an extreme case, the discovery of a novel class of antibiotics as natural products can lead to the chemical synthesis of analogues for application, rather than their semi-synthesis from a natural antibiotic; the monobactam aztreonam (Cimarusti & Sykes 1984) and the thienamycin derivative imipenem (Kropp *et al.* 1985) are notable recent examples. Thus chemistry will continue to be a fundamental tool in antibiotic development as long as new antibiotics are sought.

(*c*) *Casting the screening net wider*

(i) *Unusual groups of antibiotic producers*

Although many classes of antibiotics have been found to be produced by organisms belonging to widely separated taxonomic groups – e.g. penicillins and cephalosporins in moulds and actinomycetes, and aminoglycosides in actinomycetes and bacilli – the chance of discovering hitherto unknown antibiotics is likely to be greater the wider the taxonomic net is cast. Some time ago, interest switched to the so-called 'rare' actinomycetes, i.e. members of genera other than *Streptomyces* (Bérdy 1980). Although streptomycetes seem to produce members of every antibiotic class also produced by other individual genera (Okami & Hotta 1988), different members of some antibiotic classes may be confined to particular organisms. In any event, the strategy of screening 'unusual' actinomycetes has had some recent successes, yielding, for example, teicoplanins from *Actinoplanes* (Parenti *et al.* 1977), aridicins from *Kibdelosporangium* (Sitrin *et al.* 1985) and parvodicins from *Actinomadura* (Christensen *et al.* 1987). (There has also been a tendency to re-classify some known antibiotic-producing members of the genus *Streptomyces* in 'minority' genera, such as *Saccharopolyspora* for the erythromycin producer and *Amycolatopsis* for the rifamycin producer!)

A dramatic success was achieved following the application of novel screens for β-lactam

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antibiotics (see below) to unicellular bacteria, organisms not hitherto noted as antibiotic producers; the result was the discovery of the important monobactams (Sykes *et al.* 1981; Imada *et al.* 1981).

The general correlation (albeit imperfect) between a life cycle with an alternation of different morphological cell types and antibiotic production, suggested to be adaptive by Chater (1984), has received further support from the discovery of a wide range of chemical classes of antibiotics, including several novel types, from the myxobacteria (Reichenbach *et al.* 1988). According to the advocates of these bacteria, who have invested considerable effort in devising procedures for isolating them in pure culture, they are likely to become the producers of several commercially important antibiotics in the future.

(ii) *Considering the physiology of the producing organism*

There is still much to learn about the physiology of antibiotic production, and what determines the switch from primary to secondary metabolism in the filamentous actinomycetes and moulds. This gap in knowledge, with its strong bearing on the rational improvement of antibiotic fermentations, and the expectation that advances in genetics could help to plug it, recently stimulated a new initiative by the Biotechnology Directorate of the U.K. Science and Engineering Research Council, in the form of a 'Club' arrangement to increase research on these topics (Newell 1987). Meanwhile, the realization that the efficient repression of many secondary metabolic pathways by readily utilized sources of carbon (often glucose) or nitrogen (typically ammonia) or by inorganic phosphate (Martin & Demain 1980) may militate against the discovery of novel compounds, has prompted the use of ammonia-trapping or phosphate-trapping agents in screening media, leading to the discovery of several compounds that would otherwise have been missed (Ōmura 1986; Nolan & Cross 1988).

(iii) *Designing novel screens*

The last eight years have seen the introduction into medicine and agriculture of some important antibiotics whose discovery depended directly on the application of novel screening régimes. Several are β-lactams, a class of compounds which has been so successful, and so heavily studied, that one might have doubted if so much further novelty could be exploited. Two new screens added orders of magnitude more sensitivity to available detection systems. One approach used bacterial mutants hypersensitive to inhibition by β-lactam antibiotics, and led to the discovery of the monocyclic β-lactams: the nocardicins (Aoki *et al.* 1976) and monobactams (Imada *et al.* 1981). The other, which independently led to the discovery of the monobactams (Sykes *et al.* 1981), exploited the fact that the β-lactamase of *Bacillus licheniformis* requires a β-lactam for its induction; the β-lactamase produced is easily detected by a chromogenic reaction, giving a large amplification in the sensitivity of detection, added to a primary amplification at the induction step. The end result is sensitivity down to 1 ng of antibiotic. β-lactamase was also cleverly exploited, but this time as a target for inhibition, in a screen that revealed clavulanic acid (Brown *et al.* 1976). This compound, in conjunction with a β-lactamase-sensitive semi-synthetic penicillin, is used in the very successful Beecham formulation Augmentin.

Another ingenious screen, based on the specific properties of glycopeptides as inhibitors of bacterial cell wall biosynthesis, was found to be highly specific for antibiotics of this class, and revealed several novel compounds such as the aridicins, kibdelins and parvodicins. It uses a

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tripeptide (diacetyl-L-Lys-D-Ala-D-Ala) to antagonize the activity of glycopeptides against a sensitive bacterium (Jeffs & Nisbet 1988).

Use of other, more laborious, screens for elimination of nematode infestations in mice, or for the killing of green plants, yielded the very important and novel agricultural products avermectin (Stapley & Woodruff 1982) and bialaphos already mentioned in §2*a*. In the latter case the screen was actually for compounds active against a plant pathogenic fungus (Kondo *et al.* 1973); the herbicidal properties of bialaphos only emerged when the host plants died!

Perhaps the most important new concept in antibiotic screening will turn out to be the use of the products of cloned genes from pathogens or cancer cells as targets for inhibition *in vitro*. In the anti-bacterial field, *Mycobacterium leprae*, the leprosy pathogen, is impossible to grow *in vitro* and very laborious to handle *in vivo* in the mouse foot-pad assay. Some emphasis is therefore currently being placed by the World Health Organisation on the development of novel screens for anti-mycobacterial drugs by using cloned drug targets (Hopwood *et al.* 1988). Pathogenic fungi may represent another similar case. However, the main thrust for the use of cloned targets is in the search for more selective anti-tumour and anti-viral drugs. Important targets for anti-cancer agents would include cloned oncogene products (Stringfellow 1985; Sigal *et al.* 1987), while some targets for anti-viral drugs would be interference with cell infectivity at the receptor level, the functions of viral enzymes and viral assembly (Sarin 1988).

(*d*) *Genetic manipulation of the antibiotic producer*

I take for a starting point for this section an article written eight years ago (Hopwood 1981) under the title 'Future possibilities for the discovery of new antibiotics by genetic engineering' in which I considered the possible use of two quite different routes to the generation of chemical novelty by altering the genotypes of microbial cultures. In the first, the expression of 'silent' genes, the hope is to uncover an innate biosynthetic capacity to produce an antibiotic that is not normally seen, while in the second the objective is to generate a 'hybrid' antibiotic by making a hybrid organism.

(i) *Expression of 'silent' genes*

There are three examples in which novel compounds were produced by recombinants generated by conjugation between streptomycete strains, either different species or descendants of the same wild type (Schlegel & Fleck 1980; Mazières *et al.* 1981; Schupp *et al.* 1981). I believe (Hopwood 1983) that the most likely explanation for the origin of the novel compounds is that recombination led to genotypes in which potential genetic information from only one of the parents was expressed, although in the case of iremycin (Schlegel & Fleck 1980) structural information for the novel compound might well have come from *both* parents. A more recent example, in which protoplast fusion between blocked mutants of two aminoglycoside-producing streptomycetes resulted in strains that made a novel non-aminoglycoside antibiotic (indolizamycin) was also interpreted by the authors as a case of activation of 'silent' genes in one of the parental lines (Okami & Hotta 1988).

In a recent study, potential genetic information for antibiotic production was probably detected before it was subsequently expressed by mutation or physiological manipulation. Malpartida *et al.* (1987) used probes consisting of *Streptomyces coelicolor* genes controlling early steps in the assembly of the carbon chain of the polyketide antibiotic actinorhodin (*act* genes) to reveal polyketide synthase genes in a series of other *Streptomyces* strains by Southern

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hybridization. Although nearly all the known polyketide-producers showed hybridizing bands, as expected, the DNA of two of the polyketide *non*-producers studied also showed bands homologous to the probes. Both strains were subsequently shown to be capable of polyketide production, in one case when a mutant was isolated that did not make the non-polyketide antibiotic of the parent strain (*Streptomyces parvulus*: U. Keller, personal communication) and in the other after a change of culture conditions (*Streptomyces venezuelae*: L. C. Vining, personal communication).

A fascinating recent discovery, perhaps related to these findings, is that recombinant plasmids carrying segments of DNA that hybridized with the *act* gene probes (or a homologous gene from the tetracenomycin producer), which were isolated from the producers of polyethers, macrolides and anthracyclines, gave rise to antibiotics when introduced into a common host, *Streptomyces lividans* (Borell *et al.* 1989). The compounds included novel metabolites related in structure to, but distinct from, those found in the strains that donated the cloned DNA. The explanation suggested for these novel compounds was a change in the regulation of the biosynthetic genes on transfer to a new genetic background.

An intriguing case of 'silent' genetic information was reported by Jones & Hopwood (1984). Two separate fragments of DNA from the actinomycin-producing *Streptomyces antibioticus* caused *Streptomyces lividans* (and some other *Streptomyces* species) to produce the enzyme phenoxazinone synthase (PHS) which is involved in actinomycin biosynthesis in *S. antibioticus*, although the clones did not carry a structural gene for this enzyme. (A third clone, which did contain the PHS structural gene, also gave rise to active enzyme in *S. lividans*, as expected.)

Although the term 'silent gene' in all these situations may be a convenient shorthand, it is probably misleading. More likely, the genetic information concerned is in fact expressed under particular ecological, physiological or developmental conditions in which antibiotic production is appropriate, but which differ from those normally used in antibiotic screening. We should not be surprised. Most antibiotic producers, notably the filamentous actinomycetes and moulds, have complex cycles of differentiation and their colonies can even be regarded as having different 'tissues'. Tissue-specific gene expression is a general phenomenon in differentiated organisms. The challenge to the biotechnologist is to try to devise general strategies for revealing the total potential for antibiotic production that his or her painstakingly isolated strain collection possesses. Probably this will happen only after genetic and physiological studies have gone further in revealing the control of differential gene expression in antibiotic-producing microorganisms (see §3).

(ii) '*Hybrid*' *antibiotics*

The idea that novel compounds could be generated by cloning some or all of the genes for the biosynthesis of one antibiotic into a strain capable of producing a different member of the same chemical family was reduced to practice by the production of mederrhodins A and B and dihydrogranatirhodin through the transfer of *S. coelicolor* genes involved in actinorhodin biosynthesis into the producers of medermycin or dihydrogranaticin (Hopwood *et al.* 1985; Ōmura *et al.* 1986). A more recent example of a 'hybrid' antibiotic is isovalerylspiramycin, produced by cloning DNA from *Streptomyces thermotolerans*, the producer of carbomycin (which has an isovaleryl side chain), into *Streptomyces ambofaciens*, which makes spiramycin (Epp & Schoner 1988).

In these, and other examples that may be in the pipeline (Baltz *et al.* 1986), the 'hybrid'

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molecules will usually differ in comparatively simple ways from the parental compounds and have readily rationalized structures. (For the nor-erythromycins, made by transferring DNA from the oleandomycin producer into the erythromycin producer, the origin of the resulting macrolide ring was not easily interpreted (McAlpine *et al.* 1987); perhaps it arose by a perturbation of the normal programmed assembly of the polyketide – see below – rather than by the introduction of a specific enzymatic property from the donor of the DNA.) In the 'hybrid' antibiotic approach, the innate breadth of substrate specificity of particular biosynthetic enzymes is exploited by making available to them substrates that they do not normally encounter. This approach may therefore be a valuable route to the production of an identified target molecule (perhaps avoiding difficult stereospecific chemical conversions), but is rather unlikely to generate structures fundamentally different from those already known. The generation of greater chemical novelty may become possible if one can change the specificity of an antibiotic synthase by site-directed mutagenesis and/or genetic engineering. The β-lactams and the polyketides are probably suitable material for such an approach.

The key enzyme in the synthesis of the most widely used β-lactam antibiotics, the penicillins and cephalosporins (including the cephamycins), is isopenicillin N synthase (IPNS), or 'cyclase', which converts the precursor tripeptide (L-α-aminoadipyl-L-cysteinyl-D-valine, or ACV) into the fused ring molecule isopenicillin N, an intermediate in the biosynthesis of all these β-lactams. The cloning of the IPNS gene, first from *Penicillium chrysogenum* (Samson *et al.* 1985) and later from other fungi and from streptomycetes (Carr *et al.* 1986; Weigel *et al.* 1988; Leskiw *et al.* 1988), and its expression in *Escherichia coli* has made the IPNS protein available for intensive studies of its reaction mechanism and substrate specificity, leading to the generation *in vitro* of a multitude of unnatural β-lactams from analogues of ACV (Baldwin & Abraham 1988). Similar studies will also be possible with the deacetoxycephalosporin C synthase, or 'expandase', which converts penicillin N by ring expansion to deacetoxycephalosporin C (Samson *et al.* 1987). Eventually, it should become possible to produce novel antibiotics by creating unnatural 'cyclase' or 'expandase' genes by site-directed mutagenesis and re-introducing them into high-yielding strains of β-lactam producers. (It will also be necessary to ensure a supply of suitable novel substrates for the modified synthases; this may involve the feeding of unnatural precursors to a strain carrying a genetically engineered ACV synthase gene.)

The polyketides, a huge class of natural products that includes many important antibiotics – anthracyclines, tetracyclines, polyethers, ansamycins and macrolides of different types (polyenes and avermectins as well as 'classical' macrolides such as erythromycin) – are biosynthetically more complex than the β-lactams. Their synthesis proceeds via an ordered condensation of acyl esters to generate carbon chains of varying length and substitution pattern that are later converted to the mature antibiotics. This process (figure 1) has long been recognized as resembling fatty acid synthesis (Birch & Donovan 1953). There are, however, important differences. Unlike a fatty acid synthase, a typical polyketide synthase (PKS) is programmed to make many choices during carbon chain assembly: the choice of 'starter' and 'extender' units, often selected from acetate, propionate or butyrate residues in a defined sequence; the choice of using a full cycle of reduction–dehydration–reduction after some condensation steps, omitting it completely, or using one of two incomplete cycles (reduction alone or reduction followed by dehydration), which determines the pattern of keto or hydroxyl groups and the degree of saturation at different points in the chain; and the choice of

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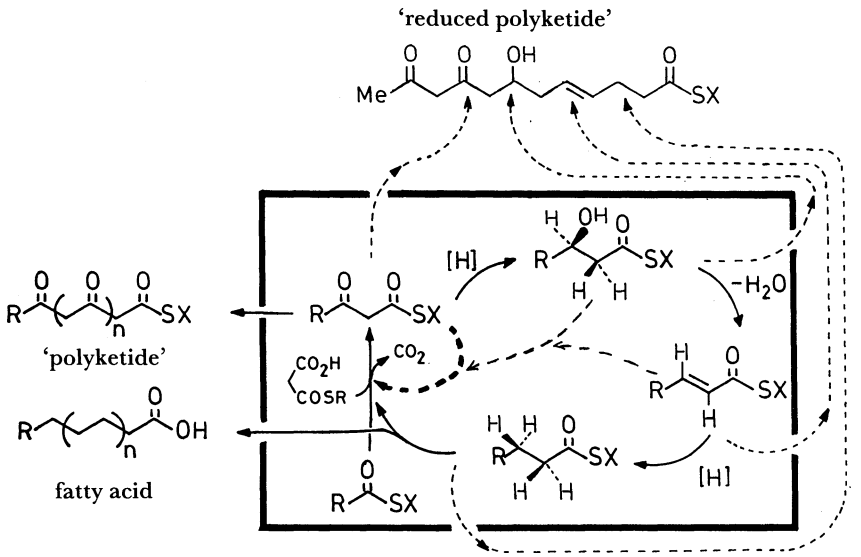

FIGURE 1. General scheme for the assembly of the carbon chains of fatty acids, polyketides and 'reduced polyketides'. After the condensation (with loss of CO_2) of each successive 'extender' unit onto the growing hydrocarbon chain, there are various possibilities. In the biosynthesis of a saturated fatty acid, a complete cycle of reduction–dehydration–reduction takes place to remove the keto group and leave an alkyl functionality. In the biosynthesis of a 'complete' polyketide, this cycle is omitted, so that a keto group remains. However, for a typical ('reduced') polyketide these two possibilities, as well as reduction–dehydration (to give an enoyl functionality) or reduction alone (to produce a hydroxyl group), can occur in a specific sequence as the carbon chain is built up; see figure 2. (From Sherman *et al.* (1988).)

stereochemistry for the substituents at many of the carbon atoms. The basis of this programming (figure 2) is essentially unknown, mainly because of the instability in cell-free extracts of the PKS complexes themselves, and of the chemical intermediates in chain assembly, but molecular genetics promises to add an important new dimension to its ultimate understanding. Following the discovery that the DNA coding for one PKS could be used as a probe to isolate others (Malpartida *et al.* 1987), sequencing of a series of *PKS* genes is revealing the primary structure of the proteins, while their over-expression will greatly aid the development of cell-free systems for biochemical study. Already, DNA sequencing has revealed, for two examples – dihydrogranaticin (Sherman *et al.* 1988) and tetracenomycin (Hutchinson *et al.* 1988) – that the PKS is a complex of several separate polypeptides, like the fatty acid synthase of *E. coli* and higher plants, rather than being a multifunctional polypeptide carrying the different catalytic functions as separate domains, like the fatty acid synthase of vertebrates (McCarthy & Hardie 1984). This may auger well for the construction, by genetic engineering, of 'hybrid' synthases capable of catalysing the production of a wide range of truly novel metabolites. Of course this will require subunits coming from different polyketide synthases to come together to form functional complexes. In this context it is promising that DNA from the milbemycin producer that is homologous to the *act*III gene of *S. coelicolor*, which appears to code for the keto-reductase involved in actinorhodin biosynthesis (Hallam *et al.* 1988), can complement an *act*III mutant of *S. coelicolor* to produce actinorhodin (Malpartida *et al.* 1987).

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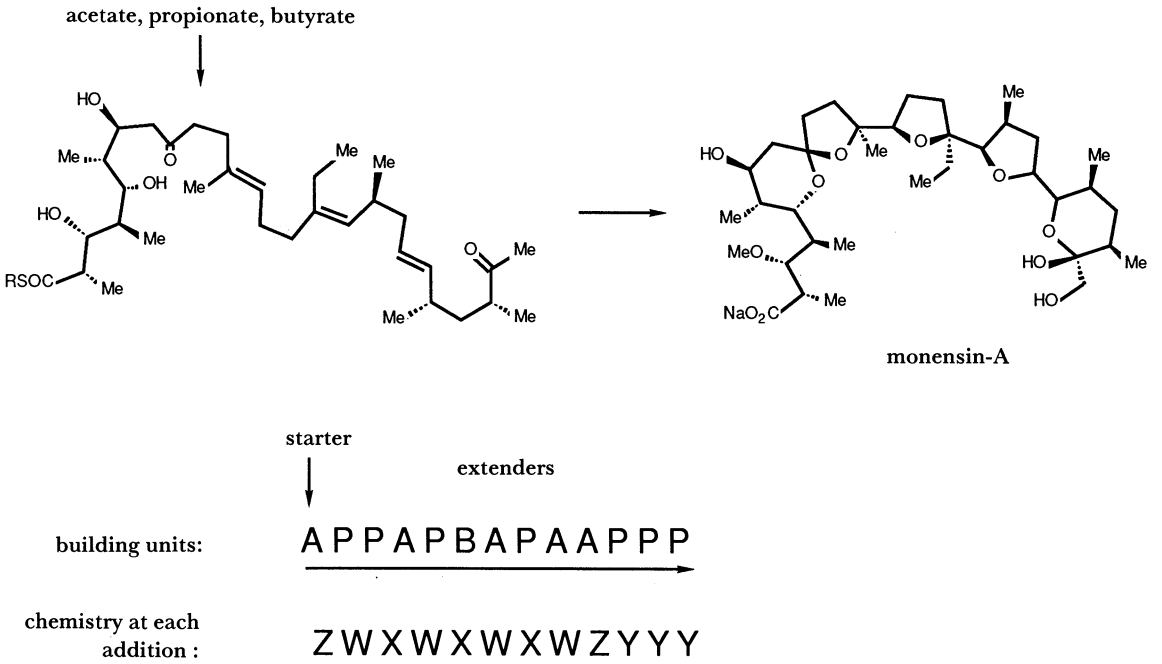

FIGURE 2. Sequence of addition of acyl residues and chemistry at each addition in the biosynthesis of the presumed 'polyketide' precursor of monensin. Building units: A, 'acetate'; B, 'butyrate'; P, 'propionate'. Chemistry at each addition: W, condensation–reduction–dehydration–reduction (to produce alkyl functionality); X, condensation–reduction–dehydration (to produce enoyl functionality); Y, condensation–reduction (to produce hydroxyl functionality); Z, condensation (to produce keto functionality). (After Sherman *et al.* (1988).)

3. INCREASING ANTIBIOTIC YIELD

(*a*) *Protoplast fusion to recombine divergent selection lines*

Although empirical mutation and screening procedures still dominate programmes for the improvement of the productivity of antibiotic-producing streptomycetes, the random recombination of different selection lines by protoplast fusion (Hopwood *et al.* 1977; Baltz 1978) has established a place in some companies. Unfortunately, documentary evidence of its utility will always be difficult to obtain, because this is shrouded in commercial secrecy, although some model studies have been described (see, for example, Kitano *et al.* 1985).

(*b*) *Overcoming metabolic bottlenecks*

Certain highly developed industrial-scale antibiotic fermentations produce, in addition to the desired end product, significant quantities of biosynthetic intermediates that are either wasted or have to be converted to the end product by more or less costly procedures. Such cases are obvious candidates for gene cloning, because over-expression of an individual gene product in the antibiotic producer – an enzyme present in limiting quantities – promises to solve the problem (Baltz 1982). Successes in this kind of situation are also likely to remain unpublished, but will probably be the first examples of practical application of cloning technology in antibiotic-producing actinomycetes and fungi, a technology that is now adequate to the task. Moreover, because of the high antibiotic titres involved, the financial return is expected to be large.

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(c) Understanding and manipulating the regulation of antibiotic biosynthesis

The genetic determination of antibiotic production is extremely complex. The information that programmes the structure of any one compound is encoded in, say, 10–30 genes, depending on the number of steps in the pathway. Over and above this, probably at least several hundred genes affect the amount of antibiotic produced under a given set of environmental conditions. Thus antibiotic titre in a fermentor is a classical continuously variable character that would require a painstaking biometrical analysis to describe (Hopwood & Merrick 1977). This is why, apart from the special cases of significant metabolic 'bottlenecks' discussed above, antibiotic 'yield' is not an ideal situation for the kind of single-gene cloning solution that would be suitable for improving the production of a useful industrial enzyme or mammalian hormone. On the other hand, molecular genetic analysis, including determination of the organization and transcriptional control of the antibiotic biosynthetic genes, is now poised to yield information that will certainly be scientifically rewarding and will probably lead also to applicable knowledge.

In the five years since the cloning of the first antibiotic biosynthetic genes from *Streptomyces*, many such genes, often sets of genes controlling whole pathways, have been cloned. These successes have revealed that clustering of such genes is probably universal (see reviews by Hopwood 1986; Chen 1989; Bibb 1988). The transcription patterns of some of these clusters are being worked out and some generalizations have emerged, including the idea that the resistance genes that often form part of the clusters are involved in the selective transcriptional activation of the biosynthetic operons (Chater & Hopwood 1989), a finding that may illuminate earlier observations that selection of mutations for increased antibiotic resistance can be a useful strategy in titre improvement (Demain 1974). Several specific regulatory genes have also been identified in the clusters, typically acting in a positive fashion, and in one case, that of actinorhodin, extra copies of the regulatory gene were found to cause a large increase in antibiotic production when cloned in the wild-type strain (Hopwood *et al.* 1986). This discovery implies that rational genetic engineering of the activator genes and their targets may be a good strategy for increasing gene expression.

In the wider context of differential gene expression, it is relevant that the regulation of antibiotic production and of morphological differentiation are intimately connected, at least in *Streptomyces* (Chater 1984; Chater & Hopwood 1989; Chater *et al.* 1988; Hopwood 1988). A notable and unprecedented recent finding is that the product of a *S. coelicolor* gene, *bldA*, appears to be a tRNA required to translate a codon, UUA for leucine, that is very rare in the [G+C]-rich DNA of *Streptomyces* and seems to be absent from genes required for vegetative growth (Lawlor *et al.* 1987; Chater *et al.* 1988). Thus *bldA* mutants grow normally up to the stage before secondary metabolism and morphological differentiation, but antibiotics and aerial mycelia are not produced. Study of the regulatory cascades implied by the existence of such pleiotropic genes promises to throw considerable light on the global regulation of antibiotic production.

4. Conclusion

There is no doubt that study of the molecular biology of antibiotic production has become a subject of considerable academic interest that promises to provide insights into several general biological problems. One concerns the differential expression of genes under the influence of

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specific environmental signals, and in particular morphological contexts. Another, illustrated by the polyketide and β-lactam synthases, relates to the general area of protein engineering and the relation between the primary structure of polypeptides and their catalytic properties. I think that the chances are very high that some of the knowledge obtained in these studies will lead to important practical advances in the areas of antibiotic discovery and the improvement of antibiotic productivity, so that genetics will enter into a productive partnership with more traditional techniques in the antibiotics industry.

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