GENETICS OF ANTIBIOTIC PRODUCTION BY ACTINOMYCETES[1]

D. A. Hopwood
John Innes Institute, Norwich NR4 7UH, England

Several thousand different antibiotics are known to be produced by members of the genus *Streptomyces*. Increasing numbers are being found in organisms belonging to other actinomycete genera such as *Nocardia*, *Micromonospora*, *Actinoplanes*, *Streptosporangium*, *Streptoverticillium*, and several more (2, 20, 34). It would be a superhuman and largely redundant task to learn about the number and location of the structural and regulatory genes specifying all of these antibiotics, but it would be highly interesting and relevant to study in detail the genetics of a representative sample of chemically and biologically distinct types of antibiotics in order to draw general conclusions. We are still very far from being able to do this, but a fragmentary picture is gradually being built up. Much of the available information has been reviewed recently (5, 9, 13, 23). Advances in techniques of *in vivo* and *in vitro* genetic manipulation applicable to actinomycetes, which are being harnessed to analyze the genetic determination of antibiotic production, and will be used increasingly to influence it artificially, are also the subject of current review articles (12, 25). There is, too, a recent review of mutants blocked in antibiotic synthesis (26). Such mutants provide material for genetic studies, but often they have been analyzed only biochemically or used for the production of novel antibiotics by feeding them with antibiotic precursors or analogues of antibiotic moieties in the technique of "mutasynthesis" (27).

Genetic control of antibiotic production: chromosomal and plasmid genes. Plasmids (extrachromosomal genetic elements) have been postulated to be involved in the genetic control of the synthesis of a large number of antibiotics, but not in all. Usually the nature of this control is not clear; in many examples, evidence for plasmid involvement itself is still very tentative. Nevertheless, at least three patterns of genetic control are apparent: (a) clusters of chromosomal genes code for the biosynthetic pathway enzymes and plasmids are apparently not involved, either directly or indirectly, in the biosynthesis (e.g. actinorhodin in *Streptomyces coelicolor*); (b) biosynthetic pathway genes are carried on a plasmid, and chromosomal genes are not involved in controlling steps specific to the biosynthesis (e.g. methylenomycin A in *S. coelicolor*); (c) plasmids control, in some as yet undefined way, the function of chromosomal structural genes (e.g. chloramphenicol in *S. venezuelae*). A short account of these three examples will be given, while others will be summarized very briefly (table 1).

Actinorhodin: involvement of a cluster of chromosomal genes. Mutants (*act*) of *S. coelicolor* A3(2) which fail to produce the pigmented isochromanequinone antibiotic actinorhodin are readily recognized visually. A series of 76 mutants which were normal in other respects, notably in morphology, were classified by their accumulation of different pigmented precursors or shunt products by co-synthesis of actinorhodin in pair combinations of mutants and by antibiotic activity into seven phenotypic classes, six of which were placed in the most likely biosynthetic sequence (29). Probably this is an underestimate of the number of

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structural genes specifically involved in actinorhodin biosynthesis, for two reasons. First, two of the classes contained only two members, suggesting that other classes may remain to be found. Second, there was a large class of mutants which apparently failed to accumulate any precursor or shunt product and which acted in co-synthesis with other mutants only as converter. These are likely candidates for mutants in the putative synthetase responsible for initiating actinorhodin synthesis by assembly of an acetate-derived polyketide chain; by analogy with known fatty acid synthetases, such a synthetase is likely to represent a multienzyme complex of several gene products.

Representative members of all seven mutant classes were located unambiguously in a short chromosomal map interval. In the absence of high resolution genetic mapping or, better, physical analysis of the segment of DNA, we cannot be certain that these actinorhodin genes together with any others that remain to be recognized, constitute an uninterrupted cluster, but this seems at present to be the simplest hypothesis.

Two plasmids have so far been identified in *S. coelicolor* A3(2), both of them sex factors. SCP1 carries genes for methylenomycin synthesis (see below), while SCP2 is known only by its fertility properties, and as a physically characterized DNA molecule (3, 31). Neither SCP1 nor SCP2 is required for full expression of actinorhodin synthesis since $SCP1^-$ $SCP2^-$ strains produce at least as much actinorhodin as $SCP1^+$ $SCP2^+$ cultures. Thus, if actinorhodin synthesis is subject to plasmid control, we would have to postulate the existence of a third plasmid peculiarly resistant to loss; SCP1 and SCP2 are each lost with a frequency of at least 0.2 percent, but no actinorhodin negative mutant which failed to map to the chromosome has so far been found (29).

METHYLENOMYCIN A: A PLASMID-CODED ANTIBIOTIC.—Methylenomycin A in *S. coelicolor* A3(2) is so far (table 1) the only antibiotic whose specific biosynthetic pathway is known to be catalyzed by the products of plasmid-borne genes. All apparently non-pleiotropic mutations (*mmy*) leading to loss of methylenomycin A production (at least 28 mutations of 4 or 5 phenotypic classes) are linked to SCP1. Mutations at three chromosomal loci lead to lack of methylenomycin synthesis, but these are clearly pleiotropic, since they also abolish aerial mycelium and actinorhodin production (18). SCP2 is not required for methylenomycin production. The very extensive genetic and limited physical evidence for the existence of SCP1, together with incomplete information on the biosynthesis of methylenomycin A, has been reviewed elsewhere (11, 14).

CHLORAMPHENICOL: PLASMID CONTROL OF CHROMOSOMAL GENES. All mutants of a strain of *S. venezuelae* induced by uv or nitrosoguanidine treatment which produced no detectable chloramphenicol (*cpp*) were mapped to a segment of the chromosomal linkage map, along with certain mutants arising after acriflavine or high temperature treatment and accumulating a presumptive precursor of chloramphenicol, 1-deoxychloramphenicol, or only trace amounts of chloramphenicol even under optimal conditions (1). These mutants presumably identify a minmum of two structural biosynthetic genes, and probably more since chloramphenicol-producing recombinants arose with rather high frequency in certain pairwise crosses of *cpp* mutants. Other *cpp* variants, which arose frequently after acriflavine or high temperature treatment, failed to map to the chromosome. These served to identify a plasmid involved in chloramphenicol production, presumably indirectly since this class of *cpp* variant could still produce 10% of the antibiotic yield of the cpp^+ strain under optimal conditions. There is some physical evi-

TABLE 1. Genetic control of antibiotic production in actinomycetes.

Species	Antibiotic	Genetic control			Reference
		Chromosomal genes	Plasmid genes[a]	Evidence for plasmid[a]	
S. coelicolor A3(2)	Actinorhodin	Cluster of at least 7 structural genes	None known	—	29
S. coelicolor A3(2)	Red antibiotic	Cluster of at least 5 structural genes	None known	—	28
S. coelicolor A3(2)	Methylenomycin A	No *specific* genes known (only pleiotropic morphological mutations found)	SCP1 sex plasmid carries resistance and at least 4 or 5 structural genes for production	Extensive genetic data, including fertility variants, SCP1' strains and infectious interspecific transfer; some physical evidence	9 11 14
S. venezuelae	Chloramphenicol	Group of 2 or more structural genes	A plasmid controls (high level) expression of chromosomal genes for production	Non-producers after ACR or high temperature; non-linkage with chromosomal markers; some physical evidence ("flower-shaped" DNA correlated with production)	1 24
Streptomyces species 3022a	Chloramphenicol	No information	A plasmid may be involved in production	Non-producers after EB	19
S. rimosus (3 strains)	Oxytetracycline	Two chromosomal clusters of structural genes	A plasmid controls production and resistance *in one strain*	Non-producers after ACR; infectious intra-strain transfer; some physical data(?)	9
S. bikiniensis var. *zorbonensis*	Zorbamycin, Zorbonomycins	One chromosomal gene directly involved (2 others pleiotropic?)	No information	—	6
N. mediterranei	Rifamycin B	A chromosomal structural gene for the final biosynthetic step	No information	—	32
S. reticuli	Leucomycin	No information	A plasmid in involved in production and resistance	Non-producers after EB; infectious intra-strain transfer; physical analysis of plasmid DNA, including restriction patterns of deletions	30

S. hygroscopicus	Turimycin (leucomycin)	May be involved (some non-producers not converted by reinfection)	A plasmid may be involved in production	Non-producers after ACR, EB or growth in chemostat; infectious intra-strain transfer	21
S. clavuligerus	Holomycin	One chromosomal gene for production	A plasmid may control (high level) production	Non-producers after UV; non-linkage with chromosomal markers and infectious intra-strain transfer	16
S. kasugaensis	Aureothricin	Indirect evidence for chromosomal control of synthesis	A plasmid may be involved in regulation of synthesis	Non producers after ACR or high temperature; physical analysis of plasmid DNA correlated with production	23
S. kasugaensis	Kasugamycin	No information	A plasmid may be involved in production	Non producers after ACR or high temperature; some physical evidence ("flower shaped" DNA correlated with production)	24
S. fradiae	Neomycin	No information	Plasmids may be involved in production of the DOS moiety and in resistance	Non-producers after ACR; physical analysis of ccc plasmid DNA correlated with production	36
S. kanamyceticus (2 strains)	Kanamycin	No information	A plasmid may be involved in production of the DOS moiety (but pleiotropic effects on aerial mycelium formation also occurred)	Non producers after ACR, EB or high temperature	4 15
S. rimosus paromomycinus	Paromomycin	No information	Plasmids may be involved in production of the DOS moiety and in resistance	Non-producers after ACR	35
S. bikiniensis	Streptomycin	No information	A plasmid may be involved in production and resistance	Non producers after ACR or EB	33
Streptomyces species M-506	Antimycin	No information	A plasmid may be involved in production	Non-producers after ACR or high temperature; ccc DNA correlated with production	7
S. parvulus *S. antibioticus*	Actinomycin D	No information	A plasmid may be involved in production	Non-producers after ACR or novobiocin; transfer in intra-strain protoplast fusions (but high frequency chromosomal recombination also occurred)	22

[a] DOS = 2-deoxystreptamine; ACR = acridines; EB = ethidium bromide.

dence for the existence of this plasmid: *cpp*$^+$, but not the *cpp*$^-$ variants, yielded a class of "flower-shaped" DNA molecules on sucrose density gradient analysis (24), but no detailed physical characterization of the plasmid has been reported.

OTHER EXAMPLES OF CHROMOSOMAL ANTIBIOTIC GENES. *S. coelicolor* A3(2) provides a second example of a putative chromosomal cluster of structural genes identified by a group of 37 mutants (*red*), falling into five phenotypic classes, involved in biosynthesis of a red, non-polar antibiotic, distinct from actinorhodin but of unknown structure (28).

In another well-studied example, oxytetracycline synthesis in three different strains of *S. rimosus*, up to nine phenotypic classes of non-producing mutants (*otc*) have been mapped on the chromosome (reviewed in 9). Precise map locations are not clear from published information but it could be that the series of *otc* genes lie in two clusters in diametrically opposed positions on the linkage map, a situation found with many groups of biosynthetic genes for primary metabolites in streptomycetes and conceivably arising from ancestral duplication of uninterrupted clusters of genes (8).

Thus there is considerable evidence for the idea that chromosomally carried structural genes for antibiotic synthesis in actinomycetes show extensive clustering. In each of the other cases in which chromosomal genes for antibiotic synthesis have been mapped (holomycin, rifamycin B, zorbamycin: table 1), only a single locus has been found so far to be specifically involved in the relevant biosynthetic pathway. Mutations at two other loci in *S. bikiniensis* var. *zorbonensis* probably had pleiotropic effects on zorbamycin synthesis.

OTHER EXAMPLES OF PLASMID INVOLVEMENT. The remaining entries in table 1 include, at one extreme, several in which evidence for plasmid involvement is minimal, since only the least definitive of the possible classes of evidence for plasmid control of a phenotype (9) is available; loss of antibiotic production after treatments which, in some other organisms, result in plasmid "curing". Clearly, these examples, in particular, are in need of further study to confirm the involvement of plasmids.

At the other extreme, there can be little doubt that a physically well-characterized plasmid is needed for detectable expression of antibiotic production. In *S. reticuli*, which is probably the best example, plasmid deletions were correlated with loss of antibiotic production, so the conclusion of plasmid involvement did not rely simply on a failure to detect plasmid DNA in non-producing variants (30). Other examples fall between these two extremes in the strength of the evidence for plasmid involvement in antibiotic production but they share with the extremes a lack of firm evidence on the nature of the genetic control over antibiotic synthesis exerted by the putative plasmids, whether this is due to plasmid-linked structural biosynthetic genes, to plasmid-linked regulatory genes, or to an indirect pleiotropic effect of plasmid loss, perhaps reflecting an interference with some central metabolic or developmental control, or possibly causing an alteration in the properties of the cell membrane (23).

It would probably not make good evolutionary sense for chromosomal genes to be controlled by plasmid-linked specific regulatory genes of the type known for certain primary metabolic and catabolic pathways in *Escherichia coli*. Thus, when we speak of plasmids regulating the expression of chromosomal structural genes for antibiotic production, some more general control of metabolism seems likely. For example carbon or nitrogen catabolite repression (17) or, perhaps, regulation of export from the cells may be involved. On the other hand, the

sporadic occurrence of certain antibiotics in diverse taxonomic groups (9, 24) is suggestive of plasmid-borne structural genes. The deoxystreptamine moiety is a particularly good example (15, 36) since it is a molecule found only as part of the structure of aminoglycoside antibiotics. Moreover, in several examples (table 1), aminoglycoside-non-producing variants arising after treatment with presumed plasmid-curing agents produced the relevant antibiotic when supplied with deoxystreptamine, indicating that a failure to synthesize this particular moiety was the only defect in such variants. It is to be hoped that the imminent availability of new techniques for the genetic analysis of antibiotic-producing actinomycetes (10, 12, 25) will soon provide answers to some of the questions about the genetic control of antibiotic production which puzzle us today.

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