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Advances in *Streptomyces coelicolor* Genetics

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INTRODUCTION

Since the last detailed reviews of the methodology and results of genetic studies with *Streptomyces coelicolor* (51, 87), a number of advances in our understanding of genetic phenomena in this organism have been made. Most recent research has been in three areas: the fertility system; colonial differentiation; and the interactions between bacteriophages and streptomycetes. There have been some radiobiological studies and preliminary investigations of the genetic and biochemical organization of a few pathways. A number of improvements in the techniques of mutagenesis, mutant isolation, and genetic analysis have also been made. The previous review (51) describes most of the basic methods of genetic analysis that are still in routine use in this laboratory. The practical details will not be repeated here; thus the previous article (51) should be consulted in conjunction with the present review by anyone wishing to become familiar with the practical manipulation of the organism. However, it may

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be useful to recapitulate here the main features of the genetic system of the organism as a background to the description of the fertility system which is introduced historically in a later section.

Genetic transfer in *S. coelicolor* occurs by a process which is described as "conjugation" because it results in the transfer of long segments of linked genes; but this does not necessarily imply a close structural analogy with conjugation as it occurs in gram-negative eubacteria. However the process resembles that in *Escherichia coli* and its relatives in leading to the production of merozygotes, each containing a complete (circular) chromosome from one parent and a fragment of variable length from the other. In experimental genetic studies, crosses are made by mixing spores of two genetically marked strains on solid media. Mating occurs between the hyphae produced after germination of the spores, and the results of the mating process are studied by analyzing a sample of progeny spores (or occasionally of hyphal fragments produced by mechanical disruption of the growing mat of mycelium) later produced on the mixed mycelial culture. The spores themselves do not mate so that, if mating in liquid media were to be employed, as is almost invariably the case in gram-negative eubacteria, the difficulties of handling the heterogeneous mycelial aggregates that result from spore germination would have to be solved, and this has not so far been seriously attempted.

The spores harvested from the crosses include recombinants and, usually, members of both parental classes produced asexually. In most cases the latter are in a large majority, and it is feasible to study the recombinants only by selecting various genotypic classes on media on which neither parental type can develop. In certain combinations of mating types, however, recombinants are very frequent, and may even make up virtually the entire spore progeny of the cross; in these circumstances it is useful to recover a sample of the total progeny as colonies on nonselective medium.

Much of the previous review (51) was devoted to a consideration, in formal genetic terms, of the events following merozygote formation, and a hypothesis was developed, which has not been disproved, that two alternative pathways are open to an individual merozygote, depending on whether even or odd numbers of crossovers occur between the chromosome fragment and the region of the whole circular chromosome with which it is homologous. Even-numbered crossovers generate circular, haploid recombinant genomes directly, whereas odd-numbered crossovers lead to terminally redundant, and therefore partially heterozygous, genomes. Subsequently, further opportunities for crossing over occur within the duplicated regions of such genomes, leading finally to the production of haploid recombinants. However, when a heterozygous genome is included in a spore that develops into a colony on selective medium, the colony contains the still heterozygous descendants of the original genome, and later a variety of haploid recombinants arising from them by crossing over at various points within the diploid region; such colonies are called heteroclones (59, 91), and these were discussed in detail in the earlier review (51).

In the section of the present review dealing with the fertility system, the characteristics of the merozygotes in various combinations of fertility types will be described, and the fertility types will be interpreted in terms of the presence, in autonomous or integrated state, or the absence, of a plasmid (SCP1) which, possibly in conjunction with other as yet unidentified factors, plays a role as a sex factor in *S. coelicolor*. It will emerge that there are some analogies between the fertility system of *S. coelicolor* and the well-known system of *Escherichia coli* K-12, but there are also differences, which justify the use of different terms, initial fertility (IF), ultrafertility (UF), and normal fertility (NF), to designate the fertility types of *S. coelicolor*.

In the last few years some of the techniques developed with *S. coelicolor* A3(2) have been extended to other actinomycetes. In most cases *S. coelicolor* has turned out to be a useful model for studies on other strains, in at least five of which some linkage data are now available: a wild-type (2) and a high-yielding oxytetracycline-producing industrial strain (37) of *S. rimosus*, a strain of *S. bikiniensis* producing zorbonomycin (20), a melanin-producing wild-type of a strain of *Streptomyces glaucescens* (R. Baumann, R. Hutter, and D. A. Hopwood, manuscript submitted for publication), a strain of *Streptomyces olivaceus* (72, 73); and the rifamycin-producing *Nocardia mediterranei* (T. Schupp, *personal communication*). In all five species a circular linkage map has been constructed, and in *S. glaucescens*, *S. rimosus*, *S. olivaceus*, and *S. bikiniensis* the arrangement of markers on the map shows a strong overall similarity with that in *S. coelicolor* A3(2) (2. 37, 73; R. Baumann, R. Hutter, and D. A. Hopwood, manuscript submitted for publication). In the strain of *S. rimosus* studied by Alačević (2), and in *S. glaucescens* (R. Baumann, R. Hutter, and D. A. Hopwood, manuscript submitted for publication), heteroclones of the *S.*

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coelicolor type (51, 59, 91) were found, and the general characteristics of crosses thus seemed to be the same as those of the early crosses in *S. coelicolor*. Heteroclones were not sought in the strain of *S. rimosus* studied by Hopwood and Friend (37), nor in *S. bikiniensis* (20), and they would not have been expected in the selective conditions employed; however, a difference from *S. coelicolor* A3(2) was the frequent occurrence of heterokaryons (defined as colonies growing on selective media, but segregating out only parental genotypes on subculture) in the crosses of the industrial *S. rimosus* strain. In *Thermoactinomyces vulgaris*, application of some of the procedures of *Streptomyces* genetics led not to a further example of conjugation, but to the first unequivocal demonstration in an actinomycete of transformation (54, 61). Genetic studies on actinomycetes other than *S. coelicolor* have recently been reviewed by Hopwood (52).

SOME TECHNICAL ADVANCES

Mutagenic Procedures

Mutagenic treatments in *S. coelicolor* have been greatly improved since 1967. The following procedures are currently used.

Ultraviolet light. Ultraviolet (UV) irradiation involves no special features. However, because *S. coelicolor* A3(2) and its descendants do not show any significant photoreactivation (see section on Radiobiology), all operations can be carried out under normal illumination. The dose for 1% survival varies from about 2,000 to 4,000 ergs/mm^2 depending on the strain (41).

N-methyl-N′-nitro-N-nitrosoguanidine (NTG). The spores of most streptomycetes are much more resistant to NTG than the cells of other bacteria, and so efficient mutagenesis of spores is achieved only at rather high NTG concentrations and at alkaline pH, which causes rapid breakdown of the NTG to the effective mutagen, diazomethane (28).

In a very intense treatment, incubation for 2 h in 0.05 M tris(hydroxymethyl)aminomethane-maleic acid buffer at pH 9 containing 3 mg of NTG per ml is used; a milder treatment might involve 1 mg/ml for 1 h.

Near-ultraviolet light in the presence of 8-methoxypsoralen. The procedure was adapted with little change (98) from that described for *Aspergillus nidulans* (86). Although different wild-type streptomycetes vary markedly in sensitivity, many differently marked derivatives of *S. coelicolor* A3(2) have (with a few exceptions) shown a reproducible sensitivity: survival of 1 to 5% after 10 min of irradiation under our standard conditions (98; V. Najfeld, *personal communication*). This treatment should give 3 to 5% auxotrophs among survivors. If a more intense treatment is required, irradiation can be continued; after 20 min, survival of A3(2) in one experiment was 0.001%, with 9% auxotrophs (98). 8-Methoxypsoralen plus near-ultraviolet light has the advantage for a general-purpose mutagen of efficient mutagenesis without, apparently, causing clustered mutations.

Comutation. Following the discovery that NTG tends to cause groups of clustered mutations in *E. coli*, probably as a result of its preferential action at the chromosomal replication fork (39), Randazzo et al. (80) investigated the possibility that *S. coelicolor* clones selected, after mutagenesis, for reversion of a marker might show an enhanced frequency of forward mutations at nearby loci. They found a high frequency of forward mutations at sites closely linked to *hisA* among NTG-induced *hisA*$^+$ revertants. This discovery opens the way to the selection of new mutations in particular regions of the linkage map.

Mutant Isolation

Some additional classes of mutants have been isolated and studied since 1967: morphological mutants with defects in sporulation, mutants resistant to rifampin or to phages, and those unable to utilize carbon sources.

The main new class of morphological mutants includes those with a white colony phenotype. Since the normal spore color in *S. coelicolor* is pale grey, it is possible to recognize visually, by their white colonies, mutants (called *whi*) defective in sporulation (17, 60; A. C. McVittie, manuscript submitted for publication), even on plates carrying many hundreds of colonies.

The isolation of induced rifampin-resistant (K. F. Chater, manuscript submitted for publication) and streptomycin-resistant mutants (D. A. Hopwood and H. M. Wright, unpublished data) has emphasized the need to allow a period of growth after mutagenesis and before plating on medium containing the inhibitor, to allow for the occurrence of segregational delay and/or phenotypic lag if induced drug-resistant mutants are to be recovered. This is, of course, not so easily arranged in a mycelial organism as in a unicellular one, but it can be done in a number of ways. A period of incubation of 6 h or more in liquid medium may be allowed before plating on agar containing the drug. If excessively clumped growth occurs, this can be dispersed by brief sonic treatment before plating. Alternatively,

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mutagenized spores may be plated directly on cellophane disks laid on agar medium lacking antibiotic, and the disks transferred, after overnight incubation, to fresh medium containing the antibiotic. Not only was the yield of rifampin-resistant mutants greatly increased by this last procedure, but it had the added advantage of ensuring the independence of mutations in different colonies isolated from the same series of platings (K. F. Chater, manuscript submitted for publication).

Dowding (J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972) found that mutants resistant to lysis by a virulent phage, VP11, could not be isolated by direct plating in the presence of the virus. This resulted from the very frequent occurrence of phenotypically resistant, but genotypically sensitive, growth after incubation long enough for resistant mutants to develop into visible colonies. The problem was overcome by plating spores with VP11 on minimal medium (supplemented with 4 mM calcium nitrate), collecting the progeny spores after 5 to 6 days, and repeating the plating with phage five more times. On testing single colonies developing after this procedure, 1 out of 120 was found to be genotypically resistant. In this way a mutation, *pel-1* (*p*hage *el*even resistance), was obtained whose segregation in crosses could be readily followed (Fig. 1).

Strain A3(2) and its derivatives grow considerably on agar media containing salts, but with no added carbon source (96). This property of agar utilization made it difficult to recognize mutants unable to grow on particular carbon sources by simple plate tests. Recently, by plating mutagenized spores on minimal medium containing glucose and replica plating to medium without glucose, it has been possible to isolate mutants that fail to grow without an added carbon source, and the mutations in two of these have been mapped to locus *agaA* (D. A. Hopwood and H. M. Wright, unpublished data). By using strains carrying *aga* mutations, the ability of the wild type to grow on various carbon sources could be assessed, and mutants defective in the utilization of various sugars could then be isolated (K. F. Chater and F. Flury, unpublished data).

Crossing Techniques

Normal crossing. Crosses are performed essentially as described previously (51) by mixing spores of complementary parental strains on solid media. The only modifications apply to "ultrafertile" crosses (see under The Fertility System) which require a reasonable balance of the two parents in the cross and an adequate concentration of each in the inoculum to give rise rapidly to confluent growth on the crossing medium. With well-sporulating parent strains it is enough to suspend spores by gently rubbing a small area of a culture with a loop containing a drop of water and mixing a loopful of each parent carefully on a moist slant of complete medium. When one or both parents sporulate poorly, material from one or more slants are suspended, centrifuged, and resuspended in a small volume of water to obtain a sufficiently dense inoculum. Some of our ultrafertile crosses, and others in which reproducible estimates of recombination frequencies were sought, have been made on plates rather than in tubes because of the greater ease with which uniform balanced crosses could be obtained; however, for most purposes crosses on slants are completely satisfactory.

Plate-crossing. Because of its compact mode of growth, *S. coelicolor* replicates very cleanly by velvet pads, and patches retain the same size and shape through several cycles of growth and replication. This property is exploited in the procedure known as "plate crossing." This technique, first introduced by Sermonti and Casciano (89), is invaluable in allowing the performance of large numbers of crosses quickly when only limited information is required from each cross. It is particularly valuable in assessing the fertility of large numbers of colonies or strains when mated with a common tester strain. The colonies from a suspension to be tested are grown on a nonselective medium, or preexisting strains are streaked in patches on such a medium. When sporulating, these cultures are replica plated with velvet to plates of complete medium on which "lawns" of 10^7 to 10^9 spores of the tester have been spread. The resulting "plate crosses" are incubated for a few days until they sporulate, when they can be replicated to one or a series of selective media on which various classes of recombinant progeny are recovered.

The technique of plate crossing is suitable for rapid genetic studies of large numbers of phenotypically similar strains. An orderly array of up to 20 patches of the strains is plate crossed with a tester strain, and the progeny are replicated to medium selecting a suitable population of recombinants. Classification of this population in respect to the mutation(s) under test is achieved by visual inspection or by further replication.

Such techniques have been useful in the

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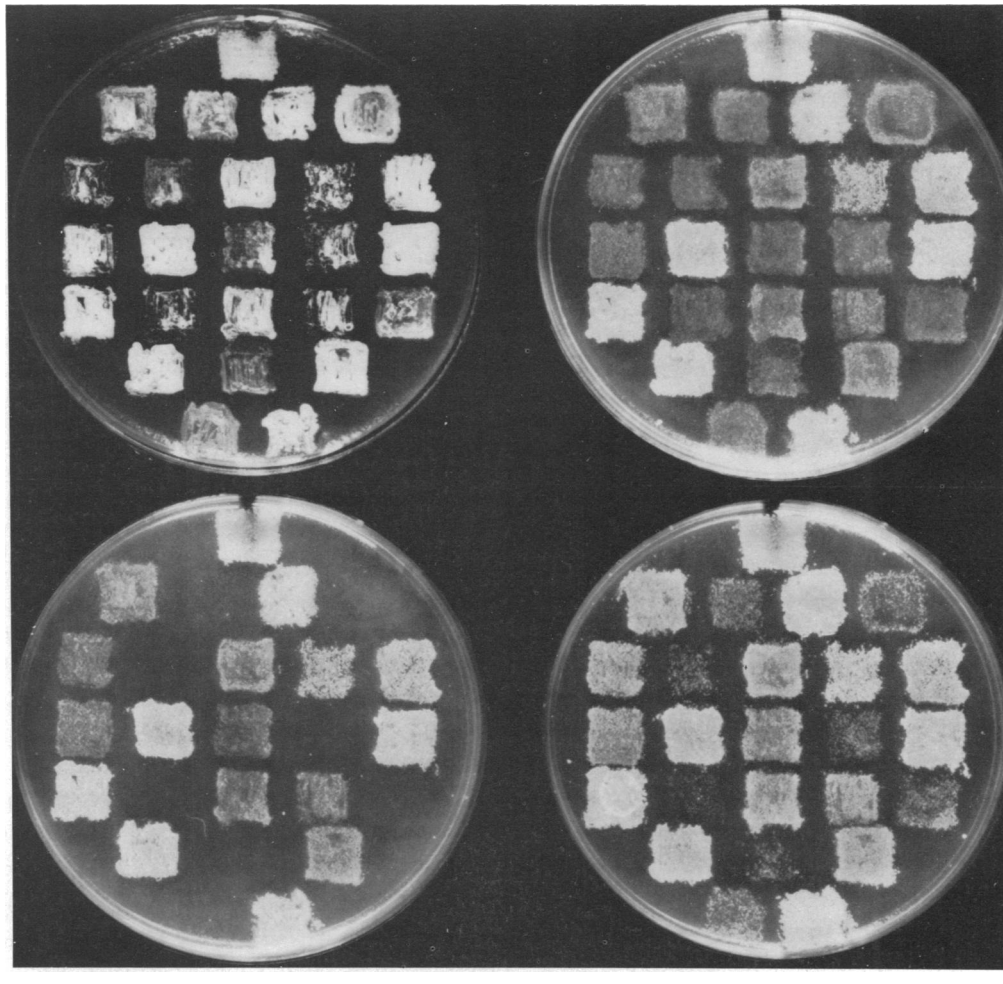

FIG. 1. *Phenotypic and genotypic resistance to phage VP11. Segregants from a cross of a strain carrying pel (phage eleven resistance) with a pel⁺ strain were inoculated on a master plate (top left) which was replica plated to new plates without VP11 (top right) and with VP11 (bottom). The bottom left-hand picture shows a clear segregation of pel and pel⁺ after 18-h incubation of the replica. By 42 h (bottom right) a considerable growth of phenotypically resistant colonies from the genotypically sensitive pel⁺ patches is seen.*

identification of recombinant strains carrying a *whiB* mutation in addition to a *whiG* or *H* mutation (K. F. Chater, unpublished data) and in the rapid identification of rifampin-resistance (*rif*) mutations differing from the common *rifA* class in not being closely linked to the *strA* locus (K. F. Chater, manuscript submitted for publication). A further adaptation of the technique was in a rapid complementation test for *uvs* mutants (43). Judicious choice of fertility type of the strains to be tested was important in all of these experiments to ensure suitable yields of recombinant colonies.

The cellophane transfer technique. The cellophane transfer technique (88), described in detail by Sermonti (87), has been applied both in *S. coelicolor* (88) and in *S. rimosus* (2). A mixed spore suspension of two parents is plated on cellophane disks over complete medium at a density just sufficient to give confluent growth after incubation for 24 h. One parent is auxotrophic and streptomycin-resistant and the other streptomycin-sensitive, so that when, after 24 h of incubation, the cellophane is transferred to selective minimal medium containing streptomycin, further parental growth is prevented. During the next few days minute tufts appear and can be isolated individually and analyzed in the same way as heteroclones (59). Probably they are not individual hetero-

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clones, but localized areas in which merozygotes have been produced, singly or in small groups, and have given rise to recombinant progeny, doubtless including heteroclones (51). It seems to us that this disadvantage renders the technique of dubious value in most studies where the objective is to select and analyze heteroclones; however, it can contribute useful information when growth of the mixed culture is to be interrupted at particular time intervals (88).

Phage Methods

Methods for studying phages of *S. coelicolor* were described by Lomovskaya et al. (68, 69) and Dowding (33).

Media. Lomovskaya et al. (69) used a peptone broth for propagation and a corn steep liquor agar for assay of ϕC31. Dowding (33) found that nutrient broth and nutrient agar supplemented with glucose and calcium ions were satisfactory for phage propagation, dilution, and assay and adopted them because of their simplicity. Nutrient broth (Difco) is supplemented after autoclaving with glucose to 0.5% and calcium nitrate to 4 mM. The medium is solidified with 1.5% or with 0.5% Oxoid agar no. 3 for use as base layers and top layers, respectively.

Lysates. Lysates of the virulent phage VP11 have been prepared both from confluently lysed plates and in shaking flasks (33). Confluent-plate lysates yielded approximately 10^8 plaque-forming units (PFU) per ml and shaking flask lysates 2 to 4×10^9 PFU per ml. The temperate phage VP5 could not be propagated in shaking flasks (J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972), and lysates were prepared only by the confluent-plate method (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press).

Phage mutagenesis. Dowding (33; J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972) found the method of Studier (97) for mutagenesis of intracellular phage by NTG to be very effective for the induction in VP11 of temperature-sensitive (up to 14% of survivors) and plaque morphology mutants (up to 4%). Free VP5 was effectively mutagenized with hydroxylamine (J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972; J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press). In the conditions employed, plaque mutants increased up to at least 8 h, when survival was approximately 1%.

Growth of Liquid Cultures for Biochemical Studies

S. coelicolor, like many other streptomycetes, grows as large granular masses of mycelium in shaken liquid culture. They are formed by the aggregation of hyphae from a large number of germlings soon after germination has taken place. Consequently, it is difficult to disrupt the cells for biochemical studies, although comparatively intense treatment with ultrasound (19; P. P. Engel, *personal communication*), Virtis homogenization (82), or grinding with alumina yields extracts suitable for enzyme assays. It has been found that the addition of high concentrations of sucrose and $MgCl_2$ to the growth medium results in liquid cultures that are much less aggregated than in conventional media, and the mycelium is much more easily lysed by lysozyme (K. F. Chater, manuscript submitted for publication; T. R. Danford and J. I. Frea, Bacteriol. Proc., p. 37–38, 1970; M. E. Townsend, *personal communication*). We routinely use nutrient broth (Difco) to which is added 34% sucrose, 1% $MgCl_2$, and 0.5% glucose.

Culture Preservation

Permanent stock cultures are kept as lyophils prepared on filter paper strips by a simple procedure (53). Unfortunately, the silica gel method (76, 77), which has the advantage that a culture can be grown from a few granules of the gel while the rest is stored for future use, was found not to be reliable for *S. coelicolor* strains (53).

In genetic work with streptomycetes, particular strains from the culture collection are constantly being required when particular combinations of markers are needed in a cross or for testing the fertility type of groups of strains. Starting from a lyophil or an old stock slant it may take 7 to 10 days before a freshly cloned culture can be prepared for use. Moreover, it is sometimes useful to go back to a cross to obtain further recombinants, perhaps imposing a new selection that was not employed in the original plating. Thus the ability to store spores in good condition ready for immediate use is invaluable. Storage of spore suspensions in 20% glycerol at −20 C has recently been found to result in no detectable loss of viability over a 15-week period during which the suspensions were nine times thawed for sampling and frozen again (K. F. Chater, unpublished data).

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THE LINKAGE MAP AND MARKERS

The current linkage map of *S. coelicolor* A3(2) is shown in Fig. 2 and a list of markers is shown in Table 1. Additional details of the morphological mutations (*bld* and *whi*) are described in the section on Analysis of Differentiation and of the *uvs* mutations under Radiobiology.

THE FERTILITY SYSTEM

First Indications of the Existence of a Fertility System

The wild-type strain A3(2) of *S. coelicolor* was a derivative of Waksman's strain 3443 (96). A3(2) was a single spore isolated by Erikson (35) with the aid of a micromanipulator from a culture obtained by her from Stanier. All of our strains have been derived either by mutation or recombination from A3(2), which was obtained by D. A. Hopwood from D. Erikson in February 1955 as an old culture preserved in sterilized soil.

Recombination between genetically marked derivatives of this culture was first shown by Hopwood (45). In these early crosses the frequencies of complementary recombinant genotypes were equal, indicating statistically equal contributions of genetic material by the two parents to the progeny, but not necessarily to each zygote (46). Assuming incomplete zygotes, as we now know to be the case, each parent had an equal probability of acting either as "donor" of fragments or as "recipient", supplying its whole genome to the zygote. Later crosses, involving additional mutants and recombinants, tended to show unequal frequencies of complementary genotypes (48, 58). Some of these crosses also showed considerably greater recombinant frequencies than those found in the early crosses (D. A. Hopwood, Ph.D. thesis, Cambridge Univ., Cambridge, England, 1958). These findings were noted at the time, but their significance became apparent only later (102).

The first attempts to investigate a possible fertility system were reported by Sermonti and Casciano (89), who were working with a set of strains derived from our culture collection. They classified certain of these strains as R^- (R = recombination) because they were intersterile, or nearly so, whereas other strains were designated as R^+ because they were fertile with each other and with the R^- strains. A fertility factor, R, was postulated to be present in R^+ and absent from R^- strains, but its transmissability from R^+ to R^- and its state in the R^+ strains were unclear. Sermonti and Casciano found evidence that, in an $R^+ \times R^-$ cross, nonselected alleles of the R^- parent tended to be inherited preferentially by the progeny, suggesting that the R^+ parent acted as a donor of an incomplete genome to the R^- strain; however, because knowledge of the linkage map was incomplete at that time, a clear picture of the constitution of the zygotes in the crosses, and therefore the relative roles of the two parents, could not be deduced.

In subsequent papers from the same laboratory, a closer analogy between R^+ and R^- *S. coelicolor* strains and F^+ and F^- cultures of *E. coli* K-12 was drawn (87). The chief new evidence contributing to this interpretation came from the results of applying the newly devised cellophane transfer technique to the study of the products of $R^+ \times R^-$ crosses (88). It was found that, when the R^- parent was resistant to streptomycin and the R^+ parent was sensitive, the longer the period of growth of a cross before transfer to selective medium containing streptomycin, the greater the proportion of tufts that contained the R^+ as well as the R^- allele at a particular locus. The results were interpreted as indicating a progressive transfer of chromosome from the R^+ (donor) to the R^- (recipient) strain in a way reminiscent of that in *E. coli* K-12.

Characterization of the NF, UF, and IF Fertility Types

In an attempt to clarify the fertility situation in *S. coelicolor*, Hopwood et al. (56, 57) set out to isolate strains of altered fertility after mutagenic treatments, rather than relying on spontaneous variations that might have arisen by chance in the stock culture collection. Because our stocks had not shown any evidence of intersterile strains, two strains were chosen at random in respect of fertility; that is simply because they were complementary in respect of a convenient set of markers. One was irradiated with UV and plated, and when the colonies were well grown they were replicated to complete medium spread with a dense spore suspension of the complementary strain. The resulting plate crosses were subsequently replicated to selective medium to reveal the fertility of each colony of the irradiated strain when crossed with the common tester strain; thus, the technique was the same as that used by Sermonti and Casciano (89), except that a large population of new colonies, rather than a small group of preexisting strains, was tested. It had already been shown by Hopwood (51) that each zygote

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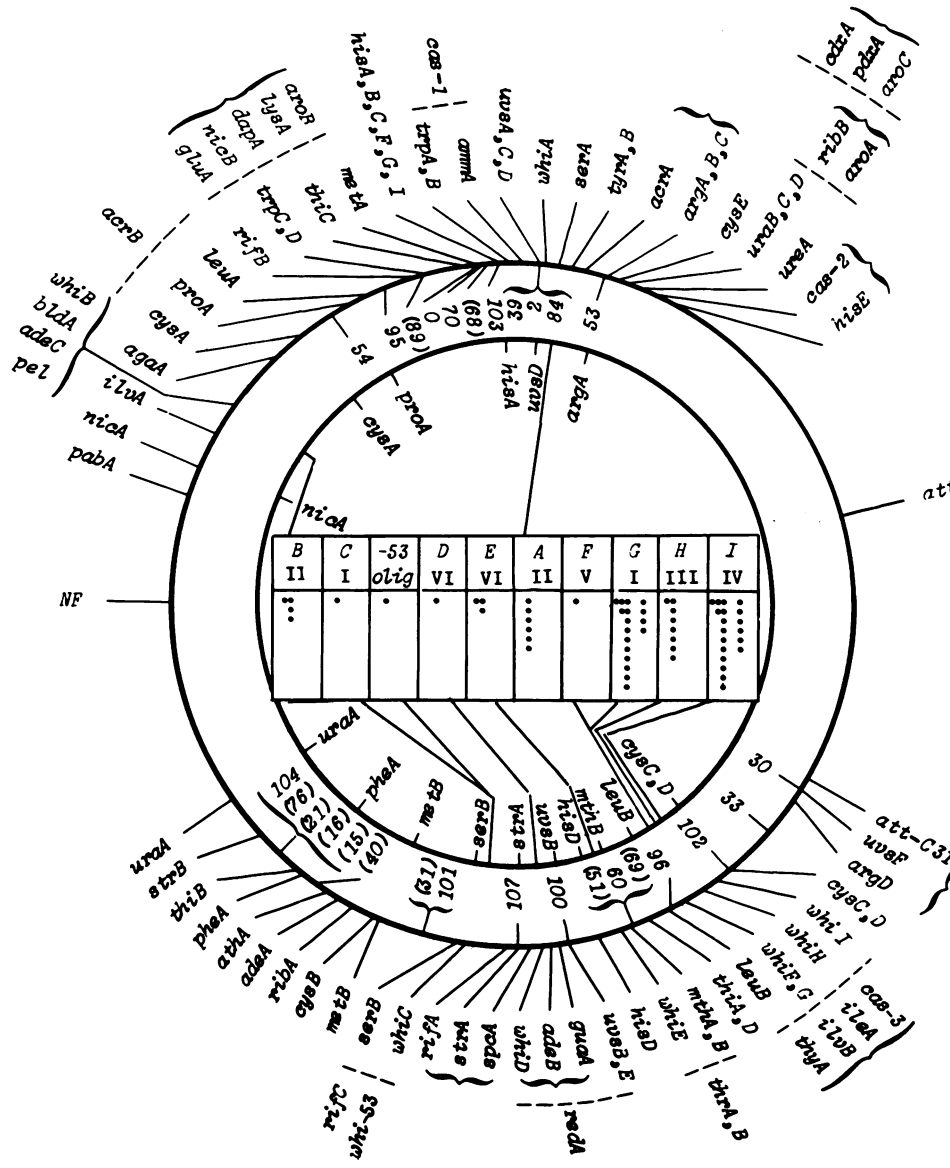

FIG. 2. *Genetic map of S. coelicolor. Outer circle: linkage map of all genetic markers mapped to date. For locus symbols, see Table 1. The order of bracketed markers, and the location of markers outside broken lines with respect to those inside the lines, is unknown. Numerals inside the outer circle are allele numbers of temperature-sensitive mutants (50), those in brackets having since been lost. Inner circle: linkage map of sporulation mutations (whi) and reference markers used to map them. Within each box is given a capital letter indicating the whi locus designation, a Roman numeral indicating the predominant phenotype of mutants of that locus, and a number of dots indicating the number of mutations identified for the locus. Two or more adjacent dots indicate mutations which may have originated from the same clone.*

in a "normal" cross contained a complete chromosome from one parent and a fragment from the other, each parent contributing the fragment to some zygotes and the complete chromosome to others. In looking for strains of altered sexual capabilities, the disposition of the selected alleles was such that the irradiated strain was obliged to contribute three markers at widely separated loci to the progeny, whereas the tester strain contributed a single marker.

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TABLE 1. *List of markers*

Locus	Alleles[a]	Characteristics	References[b]
acrA	*acr-9*, [*50*]	Resistant to acriflavine	49
acrB	*acr-3*	Resistant to acriflavine	90
adeA	*ade-3, 7, 22*, [*14*], [*15*], *h3*	Requirement for purines	49, 58
adeB	*ade-h2*	Requirement for adenine	51
adeC	*ade-f2*, [*v10*]	Requirement for purines	A
agaA	*aga-7*	Unable to utilize agar as carbon source	B
ammA	*amm-1, 2, 3, 4, 5*	Unable to utilize nitrate	49
argA	*arg-1*	Requirement for arginine, citrulline or ornithine	46, 48
argB	*arg-t74*, [*2*]	Requirement for arginine	50
argC	*arg-4*	Requirement for arginine, citrulline or ornithine	51
argD	*arg-v8*	Requirement for arginine, citrulline or ornithine	C
aroA	*aro-f1*	Requirement for aromatic amino acids	A
aroB	*aro-w1*	Requirement for aromatic amino acids	D
aroC	*aro-1, 2*	Requirement for aromatic amino acids	D
athA	*ath-2, 8, 10, 11, 12, h1, h2, h3, h4, h5*	Requirement for purines plus thiamine	49
att-C31	—	Attachment site for phage ϕC31	68
att-VP5	—	Attachment site for phage VP5	B
bldA	*bld-1*[*S48*]	Lacks macroscopically visible aerial mycelium (bald)	51
cas-1	*tps-56*	Requirement for "casein hydrolysate"	50
cas-2	*tps-59*	Requirement for "casein hydrolysate"	50
cas-3	*tps-91*	Requirement for "casein hydrolysate"	50
cdxA	*cdx-vk62*	Requirement for carbon dioxide	101
cysA	*cys-15*, [*17*], [*19*], [*20*]	Requirement for cysteine	49
cysB	*cys-4, 6, 22, 23, 24*	Requirement for cysteine or S_2O_3	49
cysC	*cys-3*, [*14*]	Requirement for cysteine or S_2O_3 or S_2O_4	49
cysD	*cys-5, 7, 9. 11, 13, 18*, [*16*], [*21*]	Requirement for cysteine or S_2O_3 or S_2O_4 or S_2O_5	49
cysE	*cys-24, v1*	Requirement for cysteine or S_2O_3	51
dapA	*dap-1*	Requirement for diaminopimelic acid	51
gluA	*glu-v1*	Requirement for glutamic acid	101
guaA	*gua-1, h1*	Requirement for guanine	49
hisA	*his-1, 120, 132*	Requirement for histidine (histidinol dehydrogenase)	49, 58, 78, 81
hisB	*his-2, 11, 12*	Requirement for histidine or histidinol (imidazole glycerol phosphate dehydrase)	58, 78, 81
hisC	*his-9, 10, 14, 119, 127, 128*	Requirement for histidine or histidinol (? isomerase, amidotransferase, or cyclase)	49, 58, 78, 81
hisD	*his-3, 4, 15, 121* [*h2*]	Requirement for histidine or histidinol (histidinol phosphate phosphatase)	49, 58, 78, 81
hisE	*his-6*, [*5*]	Requirement for histidine or histidinol or purines (PR-AMP 1,6-cyclohydrolase)	49
hisF	*his-116, 123*	Requirement for histidine or histidinol (? isomerase, amidotransferase, or cyclase)	78, 81
hisG	*his-8, 13, 117*	Requirement for histidine or histidinol (imidazole acetol phosphate transaminase)	78, 81
hisI	*his-129*	Requirement for histidine or histidinol (? isomerase, amidotransferase, or cyclase)	78, 81
ileA	*ile-h1*	Requirement for isoleucine	E
ilvA	*ilv-1*	Requirement for isoleucine plus valine	51
ilvB	*ilv-v2*	Requirement for isoleucine plus valine	C

[a] Brackets indicate uncertainty.

[b] Personal communications as follows: (A) E. J. Friend; (B) D. A. Hopwood and H. M. Wright; (C) A. Vivian; (D) P. P. Engel; (E) R. J. Harold; (F) P. J. Hartley; (G) J. E. Dowding; and (H) K. F. Chater.

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TABLE 1—*Continued*

Locus	Alleles[a]	Characteristics	References[b]
leuA	*leu-1*, [*3*], [*4*]	Requirement for leucine or α-ketoisocaproic acid	49
leuB	*leu-5*, *t92*	Requirement for leucine or α-ketoisocaproic acid	50
lysA	*lys-1*	Requirement for lysine	51
metA	*met-2*	Requirement for methionine	46, 58
metB	*met-3*, *4*, *5*, *6*	Requirement for methionine or homocysteine	49
mthA	*mth-1*	Requirement for methionine plus threonine	49
mthB	*mth-2*	Requirement for methionine plus threonine; or homoserine	49
NF	—	Attachement site of SCP1 in NF fertility type	57, 102
nicA	*nic-1*, [*2*], [*3*], [*6*], [*h1*]	Requirement for nicotinamide	49
nicB	*nic-v1* [*h2*]	Requirement for nicotinamide	101
pabA	*pab-1*	Requirement for *p*-aminobenzoic acid	51
pdxA	*pdx-v1*	Requirement for pyridoxine	101
pel	*pel-1*	Resistant to phage VP11	G
pheA	*phe-1*	Requirement for phenylalanine	46, 50, 58
proA	*pro-1*, *3*, [*2*], *h1*, *h2*	Requirement for proline	49
redA	*red-1*	Red pigment instead of blue/red indicator pigment	49
ribA	*rib-h1*	Requirement for riboflavin	B
ribB	*rib-1*	Requirement for riboflavin	51
rifA	*rif-1*	Resistant to rifampin (RNA polymerase)	H
rifB	*rif-37*	Resistant to rifampin (permeability?)	H
rifC	*rif-d6*	Resistant to rifampin (permeability?)	H
serA	*ser-1*	Requirement for serine or glycine	49
serB	*ser-2*	Requirement for serine or glycine	49
spcA	*spc-1*	Resistant to spectinomycin	51
strA	*str-1*	Resistant to streptomycin (high level)	46, 49, 58
strB	*str-11*	Resistant to streptomycin (low level)	F
thiA	*thi-1*	Requirement for thiamine; does not crossfeed *thiB*	49
thiB	*thi-3*, [*7*], [*9*], [*h2*]	Requirement for thiamine or thiazole	49
thiC	*thi-t106*, [*2*], [*4*], [*5*], [*6*], [*h1*]	Requirement for thiamine; crossfeed thiB	49
thiD	*thi-8*	Requirement for thiamine; crossfeeds *thiB*	50
thrA	*thr-1*	Requirement for threonine	51
thrB	*thr-2*	Requirement for threonine or serine	51
thyA	*thy-1*	Requirement for thymine (leaky)	F
trpA	*trp-w7*, *w15*	Requirement for tryptophan or indole; accumulation of indole glycerol (? tryptophan synthase α subunit)	34, D
trpB	*trp-1*, *v1*, *2*, *e3*, *e11*, *e13*, *e14* [*? 8*]	Requirement for tryptophan; crossfeed indole utilizers (? tryptophan synthase β subunit)	34, D
trpC	*trp-e10*	Requirement for tryptophan or [1-(indol-3′yl) glycerol 3-phosphate synthase]	34, D
trpD	*trp-e2*, *e6*	Requirement for tryptophan or indole; [*N*-(5′-phosphoribosyl) anthranilate isomerase]	34, D
tyrA	*tyr-t98*	Requirement for tyrosine or phenylalanine	50
tyrB	*tyr-1*	Requirement for tyrosine	51
uraA	*ura-1*	Requirement for uracil	49, 58
uraB	*ura-2*	Requirement for uracil	51

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TABLE 1—*Continued*

Locus	Alleles[a]	Characteristics	References[b]
uraC	*ura-3*	Requirement for uracil	51
uraD	*aur-1*, (*arg-3*, *7*)	Requirement for uracil plus arginine	51
ureA	*ure-1*, [3], [20]	Urease-negative	49
uvsA	*uvs-2*, *etc.*	UV sensitive; Hcr⁻ for phage VP11	33, 41, 42
uvsB	*vus-6*, [21]	UV sensitive; Hcr⁺ for phage VP11	33, 41, 42
uvsC	*uvs-7*, *etc.*	UV sensitive; Hcr⁻ for phage VP11	33, 41, 42
uvsD	*uvs-1*, *etc.*	UV sensitive; Hcr⁻ for phage VP11	33, 41, 42
uvsE	*uvs-13*	UV sensitive	41, 42
uvsF	*uvs-25*	Enhances UV sensitivity of mutations in *uvsA*, *uvsC*, and *uvsD* (other *uvs* loci not tested)	41, 42
whiA	*whi-72*	White colony; sporulation defective class II	17
whiB	*whi-70*	White colony; sporulation defective class II	17
whiC	*whi-193*	White colony; sporulation defective class I	17
whiD	*whi-16*	White colony; sporulation defective class VI	17
whiE	*whi-107*	White colony; sporulation defective class VI	17
whiF	*whi-99*	White colony; sporulation defective class V	17
whiG	*whi-71*	White colony; sporulation defective class I	17
whiH	*whi-119*	White colony; sporulation defective class III	17
whiI	*whi-6*	White colony; sporulation defective class IV	17
whi-53	—	White colony; oligosporogenous	17, H

Thus, any colony with an enhanced capability of acting as recipient of chromosome fragments would give an increased frequency of recombinants, whereas a colony with enhanced ability to donate chromosome fragments, and a reduced tendency to act as recipient, would give fewer recombinants. In the event variants of the former type were obtained (Fig. 3), and with a high and reproducible frequency; they proved to be so fertile (up to 100% of all the spores produced on the mixed cultures were recombinants), not only with the strain used as tester in their isolation, but with every one of seven other randomly chosen strains from the culture collection, that they were called UF for "ultrafertility." All other strains were distinguished, at that time, as NF ("normal fertility").

The characteristics of NF × UF crosses were described by Hopwood et al. (57). It was deduced that the NF strain contributed a population of fragments, but not a random population, to the effective zygotes (that is those able to yield haploid recombinant progeny), whereas the UF strain contributed a whole chromosome to each zygote. All the fragments from the NF parent carried the 9 o'clock region of its genome; in other words this region was obligate in the effective zygotes of such crosses. Furthermore, this region was obligatorily inherited by all viable haploid spore progeny from the cross. Because all the progeny were of NF fertility, it was postulated that the difference between NF and UF fertility might be controlled by a difference between the NF and UF chromosomes mapping at the 9 o'clock position. An alternative possibility of a cytoplasmic element in NF strains absent from UF strains and transferred with the same 100% efficiency as the chromosome in NF × UF matings could not, at that time, be excluded, but detailed analysis of the frequencies of different classes of progeny did not support the hypothesis of transfer of such an element, *without transfer of any chromosomal material*, to some progeny. Progeny clones of a genotype identical to that of the UF parent except for their NF property (i.e., the class which theoretically could have arisen either by "infectious conversion" or by chromosomal recombination leading to incorporation of a locus determining fertility type, but of no other segregating marker) had the frequency expected for a chromosomal recombinant receiving the implicated region of the NF parent. Thus there was no evidence for infectious conversion of UF to NF.

Figure 4 shows the average contribution of

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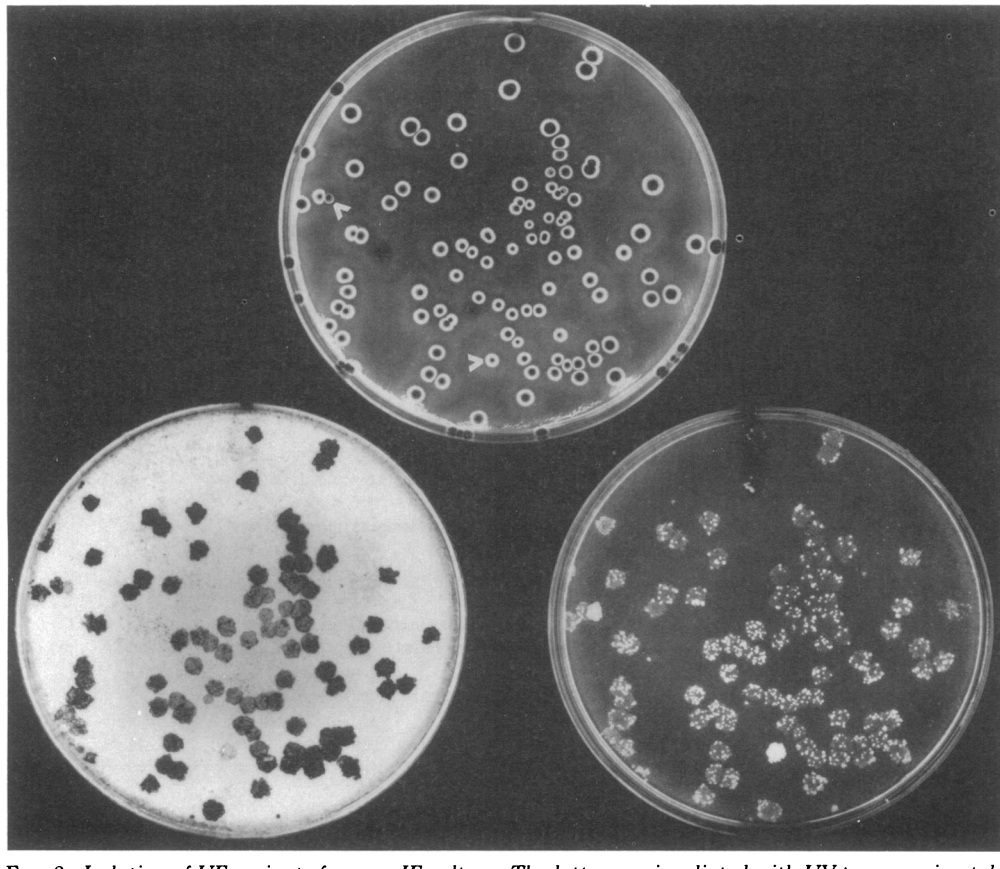

FIG. 3. *Isolation of UF variants from an IF culture. The latter was irradiated with UV to approximately 1% survival and plated (top plate). The resulting colonies were replica plated to a lawn of an NF tester strain which was incubated to produce the "plate cross" (lower left plate). This in turn was replicated to medium selecting recombinants (lower right plate). Most of the original colonies are still IF and yield a few recombinants (white) on a grey outline of leaky parental growth, but UF colonies (arrows) yield dense patches of recombinants.*

different NF markers to the haploid progeny of NF × UF matings, based on many crosses in each of which between four and eight markers were segregating. The *bidirectional* gradient of NF marker frequencies, falling in both clockwise and anticlockwise directions from the 9 o'clock position, is characteristic of such crosses. This gradient was interpreted (57) to mean that the chance of inclusion of an NF marker on a donated fragment decreases with increasing distance from the 9 o'clock region, in both directions, and it was shown that both ends of the population of fragments were in fact variable in position. Moreover, it is clear that the inheritance by haploid progeny of particular NF markers at loci in the bottom half of the map is essentially independent of the inheritance of a particular NF marker from the top half of the map (Table 2); in other words, we are probably dealing with a single population of fragments whose average midpoint is at the 9 o'clock position. By the alternative hypothesis, that there are two populations both starting at 9 o'clock and extending for variable distances either clockwise or anticlockwise, every zygote would have to receive a fragment of each type, and the proximal (9 o'clock) end of both fragments would have to be obligatorily inherited.

Although NF strains donate this *nonrandom* population of fragments to the effective zygotes in matings with UF strains, the same NF strains donate a *random* population of fragments to the zygotes when crossed with other NF strains. Moreover, in such NF × NF matings each parent provides the fragment for some zygotes, thus presumably acting as donor, and the complete chromosome for the remainder, presumably acting as recipient. This feature was dis-

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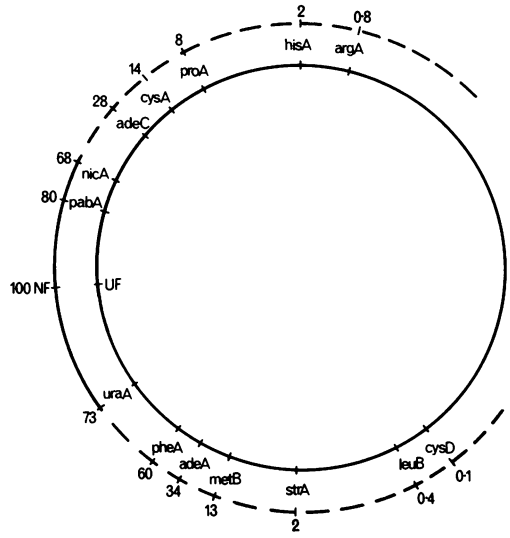


FIG. 4. *Composite diagram showing the NF allele frequency at each of a number of loci among the recombinant progeny of many NF × UF crosses. The inner circle represents the complete UF genome of the zygotes, and the outer arc indicates the chromosome fragments, always including the 9 o'clock region of the NF genome and of variable length, as indicated by the dashed lines. (From ref. 57, with later additions.)*

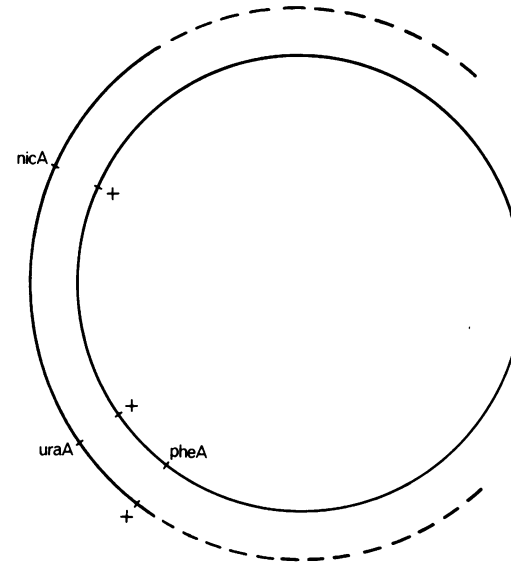
cussed in detail by Hopwood (51) by using data from crosses later shown to involve pairs of NF strains. By carrying out a total nonselective analysis of such a cross, Vivian and Hopwood (102) showed that the combined frequency of all recombinant classes in NF × NF matings could reach about 1% of all spores produced on the mixed culture.

Although the first eight strains chosen as mates for the UF strains isolated by Hopwood et al. (57) were all of NF fertility, it soon became apparent that the culture collection in fact contained strains of at least two fertility types: NF and IF. The latter was named "initial fertility" because the wild-type A3(2) belongs to this class. It turned out that, by chance, the strain that had been chosen for the isolation experiments yielding the first UF strains was IF and that this fertility type, unlike the NF, gives rise to UF strains with appreciable frequency.

At this point, it may be helpful to summarize the main features of the three fertility types in Table 3 before continuing with a description of the historical development of the subject.

A UF strain is a very efficient tester for distinguishing NF from IF strains, because in contrast to NF × UF crosses which yield *only* recombinants, IF × UF crosses yield recombinants at a frequency not usually exceeding one per 10^4 spores produced in the cross, or of the minority parental type in cases when one parent is in large excess over the other (see below). It was thus a simple matter to determine the fertility of many of the mutant and recombinant derivatives of A3(2) isolated in the early days of *S. coelicolor* genetics. Vivian and Hopwood (102) showed that all mutant derivatives of A3(2) thus far tested resembled it in being IF, and so did the earliest recombinants. However, a recombinant strain which had been isolated from among the progeny of one of the early crosses turned out to be NF; whether the change to NF had occurred as a result of the cross could not be determined, because one of the parents of the cross was no longer available for testing, and a change from IF to NF might already have occurred in this parent. From this recombinant strain, the NF character had been inherited by the majority of recombinants isolated from subsequent crosses. Nevertheless, some IF ×

TABLE 2. *Inheritance of markers by haploid progeny of an NF × UF cross*[a]



	nicA	*nicA*⁺		*nicA*	*nicA*⁺
uraA	344	147	*pheA*⁺	304	138
uraA⁺	61	59	*pheA*	101	68

[a] Data from Table 4 of ref. 57. Numbers represent the frequencies of marker combinations in a sample of progeny recovered on nonselective medium.

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TABLE 3. *Summary of the properties of the three main fertility types*

Property		Fertility type and status of plasmid SCP1		
		IF	NF	UF
Status of Plasmid SCP 1		Autonomous	Integrated at 9 o'clock region	Absent
Constitution of merozygotes in crosses with each fertility type	IF	"Random"	NF donates fragments centered on 9 o'clock; NF and IF 9 o'clock regions segregate to haploid progeny	Probably heterogeneous
	NF		"Random"	NF donates fragments centered on 9 o'clock; all haploid progeny inherit NF 9 o'clock region
	UF			"Random"
Approximate average proportions of recombinants among total spores in crosses with each fertility type	IF	0.01%	10%	0.01%
	NF		1%	100%
	UF			0.001%
Fertility type(s) of recombinants in crosses with each fertility type	IF	IF	IF and NF	IF[a]
	NF		NF	NF
	UF			UF

[a] In this case, *all* progeny (not only recombinants) are converted to IF by plasmid transfer.

NF crosses were found that had yielded both NF and IF descendants. This indication that the NF-IF difference segregated in crosses was confirmed, and it was shown that the difference, in such crosses, is chromosomally determined by alternatives at the 9 o'clock position. Any possible infectious conversion of fertility of one parent by the other in the absence of gene transfer would have been detected, but was not found (102).

Vivian and Hopwood (102) showed that the zygotes of NF × IF crosses resembled those of NF × UF crosses; the NF strain acted as a donor of fragments centered on the 9 o'clock region. The words "polarized" and "asymmetric" were used to describe this situation, the former referring to the fact that one parent functions as donor and the other as recipient (89), and the latter to the fact that the fragments represent nonrandom regions of the donor chromosome. However, from the NF × IF zygotes, in contrast to those in NF × UF crosses, there was no obligate inheritance by haploid progeny of any region of either genome; thus NF and IF segregated among the progeny. NF × IF matings were also considerably less fertile than NF × UF matings, yielding up to about 10% recombinants among all the spores derived from the crosses.

IF × IF crosses, like NF × NF and UF × UF crosses, yield mixed ("random") populations of zygotes, with each parent acting alternatively as donor and recipient. This accounts for the observation referred to at the beginning of this section, that in the early crosses, which must have been of IF × IF type, complementary recombinant genotypes usually had equal, or nearly equal, frequencies. The later departure from this state of affairs, also referred to above, must have coincided with the origin of the first NF strains. As it happened, Sermonti received some strains of each type in 1959 and 1960 and it was possible, by tracing the origin of some of his strains from our culture collection, to equate the IF and NF strains with the R^- and R^+ strains, respectively. Because R^- originally gave rise to R^+, an analogy of R^+ *S. coelicolor* with F^+ *E. coli*, as suggested by Sermonti (87), was evidently incorrect.

The next step was to study the inheritance of fertility type in IF × UF crosses, and it was shown by Vivian (100) that all of the spore progeny of such crosses, including those of each parental genotype in respect of characteristics other than fertility type, were IF. The maximum frequency of chromosomal recombinants in such crosses had already been shown (102) not usually to exceed about 10^{-4}. Thus, in an IF × UF cross, the determinant of the fertility difference was transferred at a frequency some 10,000 times higher than that of chromosomal recombination: in other words a true infectious

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conversion had been found. The putative plasmid involved was called SCP1 (*S. coelicolor* plasmid 1). Its determination of the difference between IF and UF strains explained, by plasmid loss, the high spontaneous frequency (approximately to 0.03 to 0.3%; ref. 102) with which UF strains arise in IF cultures, a frequency that was markedly increased by irradiation, but not by chemical mutagenesis. Culotta and Puglia (25) confirmed the independent inheritance of the IF determinant and chromosomal markers in IF × UF crosses, and Puglia et al. (79) confirmed the infectious conversion of UF to IF. Thus the IF strains designated R^- by Sermonti and Casciano (89) and postulated to lack a sex factor present in R^+ strains, turned out instead to harbor an autonomous plasmid and in this respect to be comparable with F^+ strains of enteric bacteria.

Evidently, NF strains could not harbor the same plasmid in the same autonomous state as in IF strains. That the plasmid was still present in NF strains was revealed through the discovery by Vivian (100) that SCP1 confers on its host strain the ability to excrete, and to be resistant to, a diffusible inhibitor of aerial growth of UF strains. Because NF strains resemble IF strains in respect to the inhibitor (Fig. 5), they must also harbor SCP1 and, if it is not autonomous, it must be associated in some way with the chromosome. The 9 o'clock position is clearly the region of association, because

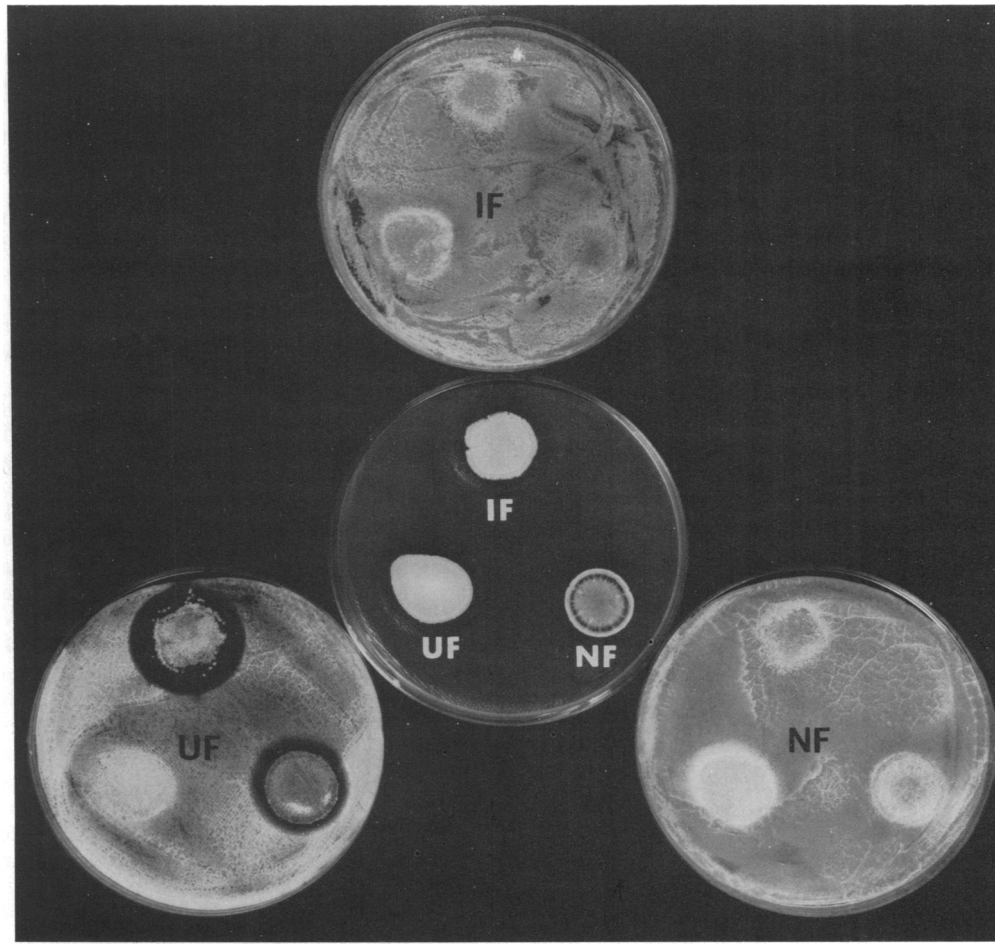

FIG. 5. *Growth reactions in pairwise crosses of IF, UF, and NF strains. The center plate carries patches of IF, UF, and NF strains, each bearing the marker pheA1. This plate was replica plated to lawns of IF, UF, and NF strains carrying the marker uraA1 to produce the "plate crosses" placed around the central plate. Note the inhibition of aerial mycelium production of the UF culture by IF and NF patches and resistance of the IF and NF cultures to inhibition. (From ref. 52).*

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this region of the NF genome is always inherited with the NF character in NF × IF and NF × UF crosses (see above).

Thus, in the sense *only* that we are dealing with strains having a chromosomally attached plasmid (NF), an autonomous plasmid (IF) and no plasmid (UF), the sexual types of *S. coelicolor* A3(2) may be compared with the Hfr, F^+, and F^- strains, respectively, of *E. coli* K-12. We will discuss some of the obvious differences between the two systems at the end of this section (see also ref. 95). Meanwhile, we should note in particular that the NF type is clearly not a close analogue of Hfr strains, because it gives a bidirectional gradient of donor marker frequencies in crosses with UF recipients; and all progeny of such crosses are donors, instead of nearly all being recipients as in *E. coli*.

New Donor Types

In order to explain the origin of all NF strains in our culture collection it has been necessary to postulate the spontaneous production of NF from IF only once, in or immediately preceding a cross made in 1956 (102). In an attempt to isolate further NF types, or any other kinds of donors that might be produced from an IF strain, Vivian and Hopwood (103) carried out the procedure that had led to the isolation of UF variants from IF cultures (see above), but this time the tester strain was UF instead of NF. In this way a strong visual selection was made for colonies within the IF population that could donate efficiently a particular allele, in this case *pabA*$^+$, to the UF culture. It was found that donors could indeed be recognized among the IF colonies. However, the *new donors* differed, usually in more than one way, from NF strains.

In the first experiments reported (103), 0.2% of the survivors of UV irradiation contained a donor clone that could be isolated. However, this is not a good estimate because of the fact that the great majority of the *new donor* types are unstable, usually markedly so. This means that they appear first not as whole colonies in the IF population, but as sectors of variable extent which have to be picked and streaked a number of times before they can be obtained in reasonably pure form. Many small sectors may be missed on the primary isolation plates or lost before they can be purified. Even after exhaustive purification, many of the *new donors* are so unstable that a plating from a carefully cloned culture may yield a majority of nondonor colonies spontaneously, and an even higher proportion after UV irradiation. Only a small minority of the *new donors* (1 out of 23 in the sample described in ref. 103) are stable.

A second feature of the *new donors* was their white colony phenotype (Fig. 6a). The strains had no morphological abnormality in spore maturation observable by light microscopy; that is, they could be compared with class VI white mutants according to the scheme of Chater (17) (see section on Analysis of Differentiation). The significance of the frequent association between ability to act as an efficient donor of chromosome segments and white colony phenotype in these strains is not known. It is not an obligate association, as shown by the frequent production of donors of normal grey phenotype from one of the white donor strains (A607); these in turn frequently gave white donors again (Fig. 6d) (103).

The most constant and novel feature of the *new donors* was their pattern of marker donation which turned out to result in a *unidirectional* gradient of marker frequency in the haploid progeny of crosses with UF strains. A particular donor region, different for different *new donors*, had a very high frequency of inheritance and the frequencies of donor markers fell gradually with increasing distance in one direction from this region, whereas there was a very abrupt cut-off of inheritance even of very closely linked donor markers in the opposite direction (Fig. 7). Thus, the merozygotes in *new donor* × UF crosses were postulated to contain a population of fragments with one end constant (that carrying the maximally donated region) and the other variable in position.

Any hypothesis on the nature of the *new donors* has to take account of the following facts: (i) these donors still contain SCP1, because they inhibit the aerial development of UF strains and are resistant to inhibition by IF strains; (ii) the frequency of inheritance of some donor markers in crosses with UF strains is as high as in NF × UF crosses; (iii) most of the donors are unstable, often very unstable, producing numerous UF variants and also some IF variants; (iv) the progeny of crosses of unstable white colony donors with UF strains have the same unstable donor state and white colony phenotype as the parent donor; on the other hand, those few donors that are stable give rise to stable donor progeny; and (v) bidirectional NF donors arise occasionally from the unidirectional donors, either from the donor culture alone or among their progeny when crossed with UF strains; conceivably, all bidirectional donors isolated from the unidirectional donors in fact arise by donor × UF mating, and this may occur within a single donor culture, because there is always a proportion of UF individuals within such a culture.

The extreme instability of some of the *new*

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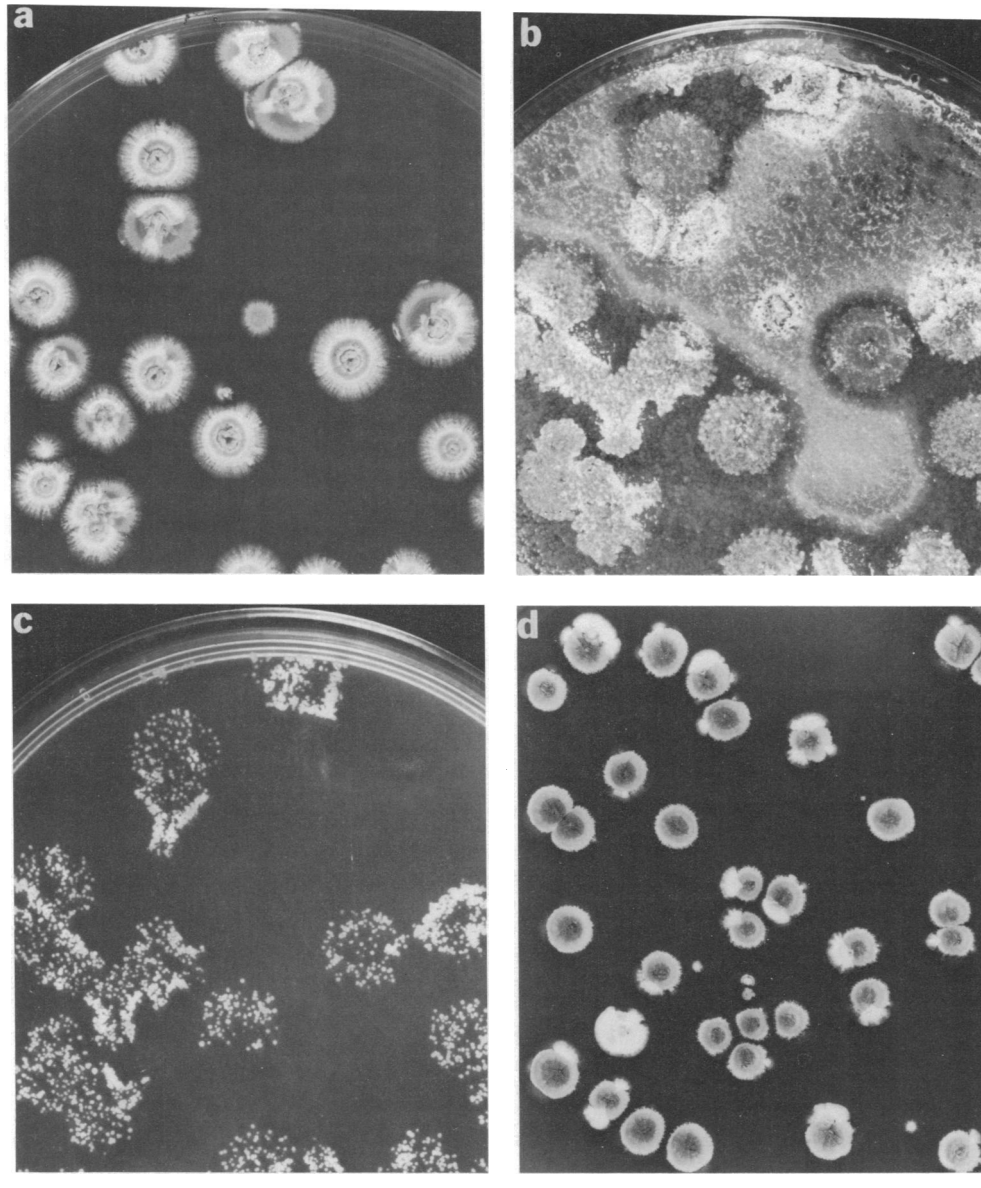

FIG. 6. *(a) Colonies of white unstable donor strain A607 producing numerous grey UF sectors. (b) Plate-cross of plate (a) on a lawn of a UF strain; note inhibition of aerial mycelium production on the background UF culture by white colonies and sectors and lack of inhibition by grey (UF) sectors. (c) Replica from plate (b) to selective medium; note recombinant production by white (donor) colonies and sectors and lack of recombinant production by grey (UF) sectors. (d) A slow-growing grey donor derivative of A607 giving rise to numerous faster-growing sectors of white donor strains.*

donors, leading to loss of ability to donate chromosomal markers efficiently, usually simultaneously with loss of SCP1, to yield UF variants suggests an autonomous state for the plasmid in the unstable donors. However, the ultrafertility of the crosses with UF recipients argues for an easy association with the chromosome. We therefore proposed (103) that the unstable donors harbored an autonomous SCP1 that had incorporated a piece of chromosome after the style of F prime strains of *E. coli* K-12 (1, 44, 63). This piece would represent a different region of the chromosome in different *new donors*, because two classes, donating a different marker with the highest frequency, were found among the 23 strains in the original

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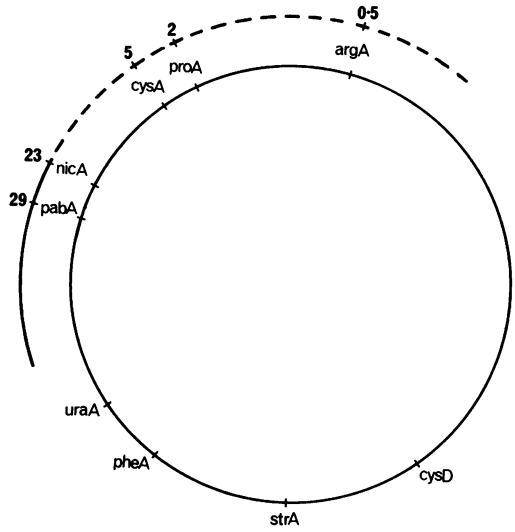

FIG. 7. *Composite diagram showing the approximate percentage frequencies of donor markers at each of a number of loci among the recombinant progeny of new donor × UF crosses. The inner circle represents the complete UF genome, and the outer arc indicates the chromosome fragments donated by a pabA⁺ donor (A607) with one constant end and one variable (dashed line).*

sample: they were called "*pabA⁺ donors*" and "*uraA⁺ donors*" after the marker donated with the highest frequency. Having, on this hypothesis, an appreciable region of homology with the chromosome, the plasmid might be expected to interact readily with it during or before its transfer to a UF recipient, possibly by "donor crossing over" (11) or perhaps by the so far poorly understood mechanism by which the F factor of *E. coli* promotes chromosome transfer by an F^+ culture (36). A *transient* interaction was suggested by the finding that the progeny of *new donor* × UF crosses resembled the donor parent in their donation properties, while being recombinant in respect of normal markers. The instability of long F′ strains of *E. coli* K-12 has been observed, but even so the extreme instability of some of the *S. coelicolor* donors is remarkable on the proposed model. The production of IF strains from the *new donors* would, by this hypothesis, perhaps occur by internal crossing over within the modified plasmid, resulting in the elimination of the inserted piece of chromosome.

This hypothesis is not applicable to the stable types of *new donors*, and probably these result from a more permanent association between the plasmid and the chromosome, as in NF strains, but presumably in a different configuration. A straightforward integration of the Hfr type is a possibility. A satisfactorily simple model to explain the behavior of NF strains is still elusive. The finding that the derivation of NF from IF strains probably involves two steps may indicate that the mechanism of chromosome mobilization in NF strains is more complex than in the unidirectional donors.

Since the isolation of the first group of *new donors* (103), an assortment of other donor types has been isolated from various IF strains (D. A. Hopwood, A. Vivian, and H. M. Wright, unpublished data). In general terms these may also be explicable by interactions between SCP1 and various regions of the chromosome. However, one recently isolated donor behaved in a much more readily interpretable fashion, and indeed this strain is clearly analogous with an F′ strain of *E. coli* K-12, because it harbors an SCP1 plasmid that has incorporated a recognized chromosomal marker. The strain has been called an SCP1′ strain (D. A. Hopwood and H. M. Wright, manuscript submitted for publication), and the particular substituted plasmid was designated SCP1-*cysB*, because it carries the chromosomal *cysB* locus. The strain was evidently a secondary (merodiploid) SCP1′ strain, rather than a primary (haploid) strain, because the plasmid was easily lost from it, giving viable UF variants.

Part of the evidence for the SCP1′ nature of this strain was the finding that, whenever the plasmid was transferred to a *cysB* UF recipient, as occurred with the same high frequency as transfer of the wild-type SCP1 plasmid from IF to UF, the progeny were converted to phenotypically Cys^+, but genotypically heterozygous (*cysB*/*cysB*⁺), strains that could lose the SCP1-*cysB* plasmid to revert to *cysB* UF derivatives. It was also shown that the SCP1-*cysB* plasmid could be transferred to another wild-type strain, 1326, to which the wild-type SCP1 had already been shown to be transferable (see below; D. A. Hopwood and H. M. Wright, J. Gen. Microbiol, in press).

Markers not borne on the SCP1-*cysB* plasmid were transferred by the SCP1′ strain to UF recipients with frequencies below 10^{-2}, and sometimes as low as 10^{-5} Thus, for some markers, they overlapped with frequencies in IF × UF crosses (see below), and it was difficult to analyze any specific pattern of marker transfer attributable to the SCP1-*cysB* plasmid over and above that due to properties of the normal SCP1 plasmid. The pattern was clearly not the same as that in the *new donors*, which were postulated to be SCP1′ strains harboring plasmids that happened to carry unmarked regions of the

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chromosome. Possibly the chromosomal insertion in the SCP1-*cysB* plasmid, which represented less than 2% of the linkage map, was too short for efficient "donor crossing over" to occur. However, much more needs to be learned about such strains before a definitive interpretation of marker transfer of nonplasmid-linked genes is possible.

Role of the Plasmid SCP1 in Fertility

UF × UF crosses, in which SCP1 is absent from both mates, are far from sterile (102). Table 4 illustrates a range of recombination frequencies in such crosses. Moreover the gene transfer in UF × UF crosses shows no evidence of occurring by a mechanism basically different from that operating in crosses involving IF or NF strains, where SCP1 is present; on the contrary, the hallmark of a conjugation system—the inheritance by the progeny of long regions of linked markers—is present in UF × UF crosses. (Dowding and Hopwood [J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press] used a UF × UF cross to map the site of attachment of the prophage of VP5 to the chromosome: see below.) Thus SCP1 is clearly not necessary for conjugation in *S. coelicolor*, and we should bear in mind that not all of the features of the conjugation observed in crosses involving IF or NF strains need necessarily be due to SCP1. In any case, an additional system controlling conjugation in UF × UF crosses evidently awaits elucidation.

IF × UF crosses are not always significantly more fertile than UF × UF crosses (102). It is not easy to obtain meaningful estimates of recombination in crosses of either type, because differences in the vigor of asexual reproduction of the various strains lead to widely different values for the proportion of recombinant spores in the cross, even if these are expressed in terms of the minority parent as in Table 4; different selections in the same cross may also lead to diverse estimates. However, a general tendency for IF × UF crosses to be more fertile than UF × UF crosses is apparent (Table 4). Moreover, comparison of the patterns of marker inheritance in pairs of IF × UF crosses involving the same groups of markers in the two parents, but with the fertility reversed, shows that SCP1 does indeed play a role in gene transfer, even when we are not dealing with strains in which it is demonstrably associated with the chromosome. In Fig. 8a the "inner" parent is IF, and it is seen to act preferentially as a donor, because the frequency of IF markers at loci such as *cys*, far from the selected marker (*pro*$^+$), are low. In Fig. 8b the "outer" parent is IF, and here there is a reverse polarization with a strong tendency for the inheritance of only a limited region of the IF chromosome around the selected marker (*str*).

TABLE 4. *Recombination frequencies in some UF × UF and IF × UF crosses*

UF parent 1		UF parent 2		Recombinants	
Genotype	No.[a]	Genotype	No.[a]	Selected markers	Frequency[b]
PheA1	1.9×10^7	*hisA1 uraA1 strA1*	9.1×10^6	*phe*$^+$ *his*$^+$ *ura*$^+$	20
pheA1	9.8×10^6	*hisA1 uraA1 strA1*	1.4×10^7	*phe*$^+$ *his*$^+$ *ura*$^+$	39
proA1 argA1 cysD18 pheA1 strA1	1.3×10^8	*ade-v10 uraA1*	4.5×10^7	*pro*$^+$ *str*	6
proA1 argA1 cysD18 pheA1 strA1	4.8×10^8	*ade-v10 uraA1*	1.1×10^9	*pro*$^+$ *str*	1
proA1 argA1 cysD18 pheA1 strA1	1.2×10^7	*hisC9*	1.4×10^6	*phe*$^+$ *his*$^+$ *pro*$^+$ *str*	340 50
proA1 argA1 cysD18 pheA1 strA1	2.0×10^7	*hisC9*	6.5×10^6	*phe*$^+$ *his*$^+$ *pro*$^+$ *str*	94 6
IF parent 1		**UF parent 2**		**Recombinants**	
pheA1	1.0×10^6	*hisA1 uraA1 strA1*	2.0×10^6	*phe*$^+$ *his*$^+$ *ura*$^+$	10
hisA1 uraA1 strA1	8.2×10^6	*pheA1*	7.8×10^6	*phe*$^+$ *his*$^+$ *ura*$^+$	87
proA1 argA1 cysD18 pheA1 strA1	7.0×10^6	*ade-v10 uraA1*	2.3×10^9	*pro*$^+$ *str*	77
ade-v10 uraA1	1.0×10^8	*proA1 argA1 cysD18 pheA1 strA1*	2.5×10^7	*pro*$^+$ *str*	19
hisC9	8.0×10^6	*proA1 argA1 cysD18 pheA1 strA1*	5.0×10^6	*phe*$^+$ *his*$^+$ *pro*$^+$ *str*	130 67
proA1 argA1 cysD18 pheA1 strA1	6.0×10^5	*hisC9*	6.2×10^6	*phe*$^+$ *his*$^+$ *pro*$^+$ *str*	1,500 17

[a] Per ml of suspension from the cross.
[b] Per 10^6 spores of minority parent.

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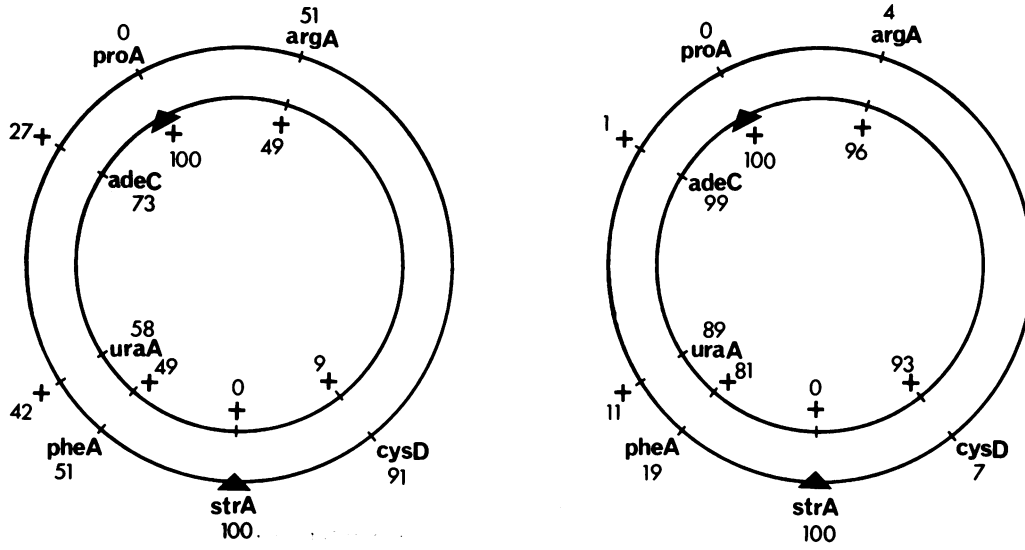

FIG. 8. *Allele frequencies (percentage) among selected recombinants (triangles indicate the selected alleles) in crosses of IF and UF strains: IF (inner) × UF (outer), UF (inner) × IF (outer). Both crosses involved strains bearing the markers ade-v10 uraA1 and argA1 cysD18 strA1 pheA1 proA1.*

As we have seen in the previous section, IF cultures contain a low frequency of donor clones able to transfer particular chromosomal regions efficiently. These presumably contribute to the marker transfer in IF × UF crosses. This interpretation is supported by the results of an experiment in which the recombinant progeny of an IF × UF cross were fertility tested by the plate-crossing technique (D. A. Hopwood and H. M. Wright, unpublished data). It was found that some of the progeny could be classified as donors, with a higher fertility against a UF tester strain than that shown by IF strains, while the remainder of the progeny were indistinguishable in this test from typical IF cultures. The two classes presumably corresponded to two possible modes of recombinant formation in IF × UF crosses.

At the upper extreme of the fertility scale, the NF × UF cross, it is clear that the presence of SCP1 associated with the 9 o'clock region of the NF genome leads to high frequency transfer of chromosome fragments carrying this region. However, it is not excluded that other classes of fragments might be transferred, but give rise to noneffective zygotes. The suppression of UF aerial mycelium by the diffusible product of SCP1 may explain the absence of UF spore progeny and conceivably, therefore, the observed obligate inheritance of SCP1, and hence of its chromosomal attachment point. Hopwood et al. (57) found a considerable proportion of the UF parent in the young substrate mycelium of NF × UF crosses, but not in the spores of mature cultures; however, a careful search among the earliest recombinants formed in such crosses, and recovered in mycelial fragments from immature crosses, failed to reveal any of UF fertility (D. A. Hopwood and H. M. Wright, unpublished data), tending to argue against the idea that UF recombinants arise, but are simply prevented by the SCP1 product from being recovered. Sermonti, Puglia, and Ficarra (92), in a detailed study of the time course of recombinant production in NF × UF crosses, also failed to find an appreciable frequency of UF recombinants even at the earliest sampling times, although the occurrence of rare UF recombinants at all sampling times, including those when mature sporulating cultures were involved, was recorded. In any case the reason for the failure of the NF parent to contribute spores to the mixed culture is unclear.

Vivian and Hopwood (103) found evidence that SCP1, carrying with it the region of the chromosome with which it is postulated to be transiently associated in the unstable *new donor* strains, is inherited obligatorily by the progeny of crosses of these donors with UF strains. Probably the same mechanism operates in these ultrafertile crosses as in NF × UF crosses.

The role of SCP1 in controlling fertility in *S. coelicolor* will doubtless be illuminated when

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mutations affecting particular plasmid functions are isolated and studied, as in the case of the F factor of *E. coli* K-12 (114). Suggestive evidence is already available to indicate that it will be possible to analyze the group of phenotypic characters controlled by SCP1, which includes at least the following: production of the diffusible inhibitor of UF strains; determination of resistance to the inhibitor; and the various aspects of plasmid maintenance and transfer. Although most of the *new donors* already isolated still possess the first two properties (103), some that we have recently isolated fail to inhibit UF cultures, although they are still resistant to inhibition by IF strains, whereas others fail to inhibit and are themselves sensitive to inhibition (D. A. Hopwood and H. M. Wright, unpublished data). If all of the donors turn out to represent various kinds of substitution of pieces of chromosome into the plasmid, those altered in production or response to the inhibitor may be interpreted as having suffered various losses of plasmid functions by deletions of varying extent. Conceivably, the extreme instability of some of the donor strains may result from loss of genes concerned in plasmid maintenance.

Transfer of SCP1 from A3(2) to Other Strains

It is characteristic of plasmids of gram-negative bacteria that they can be transferred by conjugation between strains that are not closely related: such transfer has been observed among members of the genera *Escherichia*, *Salmonella*, *Proteus*, *Pasteurella*, *Shigella*, *Serratia*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Agrobacterium*, and *Klebsiella* (4, 19, 27, 30, 66). Because nothing is known of the morphological basis of conjugation in *S. coelicolor* or other streptomycetes, we cannot say, a priori, whether conjugation between disparate streptomycetes is likely to occur. In studies of gram-negative eubacteria, great use has been made of selective plasmid-linked markers to detect plasmid transfer, even when it occurs at a low frequency: prototrophic markers in the case of substituted F factors (F's) or, more generally, drug resistances in the case of R factors. In *S. coelicolor* it is now possible to use a prototrophic plasmid-linked marker in an SCP1′ strain to detect plasmid transfer between different wild types (D. A. Hopwood and H. M. Wright, manuscript submitted for publication). Earlier, the inhibitor of UF growth determined by SCP1 (100) served to show that SCP1 can be transferred in a cross from IF strains of A3(2) to another wild-type strain of the *S. coelicolor* species group, strain 1326, converting it from a strain inhibited by A3(2) strains of IF fertility type to one resistant to inhibition and itself capable of inhibiting A3(2) strains of UF type (D. A. Hopwood and H. M. Wright, J. Gen. Microbiol., in press). One to three percent of 1326 spores harvested from such a cross had received the plasmid, as determined by a visual procedure (Fig. 9). By this procedure, we have so far failed to detect transfer of SCP1 from A3(2) to strains less closely related to it than is strain 1326, but the use of selective procedures may soon detect transfer at low frequency to such strains, just as selection allowed the demonstration of transfer of SCP1-*cysB* back from 1326 to A3(2) at a frequency of about 10^{-6} (D. A. Hopwood and H. M. Wright, manuscript submitted for publication).

Comparison of Gene Transfer in S. coelicolor with That in E. coli

The wild-type *S. coelicolor* A3(2) resembles the original strain of *E. coli* K-12 in harboring an autonomous plasmid that can be lost, can acquire an insertion of a chromosomal region, or can associate itself with the chromosome. Thus, the IF type of *S. coelicolor* may be compared with the F^+ form of *E. coli* K-12. In IF × UF crosses, as in $F^+ \times F^-$ matings, it would appear that some gene transfer may be due to donor clones within the IF culture, involving more or less permanent interaction between SCP1 and the chromosome, while some may not. This situation, in *S. coelicolor*, has not been studied in any detail, whereas in *E. coli* it has received a good deal of attention (26, 36) without a completely satisfactory explanation emerging.

Apart from these simple resemblances there are several differences between the two systems. One area of difference concerns the UF strains of *S. coelicolor*, lacking the known plasmid SCP1. These, unlike F^- strains of *E. coli*, are fertile, albeit at a relatively low level, when crossed with each other. This may mean that a second plasmid promoting conjugation and gene transfer, and present in all of our strains, remains to be identified or, alternatively, that recombination can occur in the absence of a plasmid. It may be that SCP1 itself has nothing to do with the formation of conjugation unions between the cells, a function that might be controlled by genes of a second plasmid or by chromosomal genes. However, SCP1 is transferred very efficiently in IF × UF matings (100) and in association with pieces of donor chromosome in NF × UF (57) or *new donor* × UF

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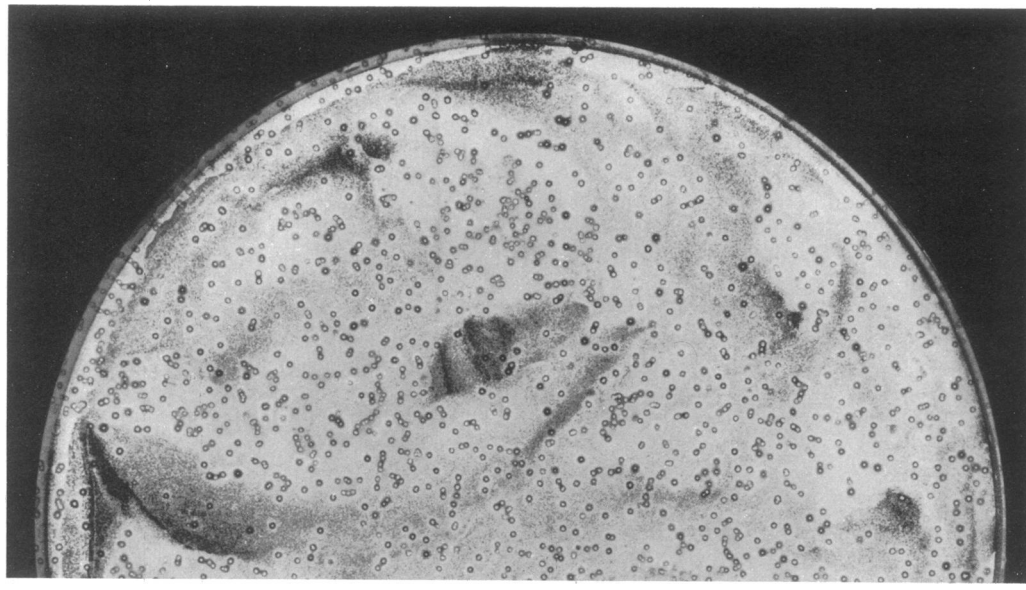

FIG. 9. *Colonies of prototrophic strain 1326 converted to IF by growth in mixed culture with an auxotrophic IF derivative of A3(2). The products of the mixed culture were plated with an excess of pure strain 1326 on minimal medium, selecting against the A3(2) auxotroph. The IF-converted colonies are surrounded by a zone of inhibition of aerial mycelium production on the unconverted strain 1326; the unconverted colonies are not seen since they merge with the background growth of strain 1326. (D. A. Hopwood and H. M. Wright, J. Gen. Microbiol., in press).*

crosses (103), and this suggests that it is responsible for its own transfer, rather than dependent on an association with some other unidentified transmissible plasmid, unless this were an extremely efficient association.

Another area of difference from *E. coli* K-12 concerns the behavior of strains capable of donating segments of chromosome. Hfr × Hfr matings display a very low fertility unless one is deliberately grown under physiological conditions leading to a female "phenocopy" (67), and then the cross is obviously polarized in favor of the phenocopy acting as recipient. In *S. coelicolor*, NF × NF crosses are very fertile, and this is not due merely to spontaneous UF phenocopies within the cultures, because the pattern of marker inheritance reveals the transfer of *random* fragments to the zygotes, rather than the special populations of fragments, centered on the 9 o'clock region, involved in NF × UF crosses. NF × IF crosses, also, are very fertile in contrast to Hfr × F^+ matings. Other differences, already discussed, concern the donation of fragments extending symmetrically on both sides of the point of interaction of the plasmid with the chromosome in NF strains and the inheritance of donor status by the recombinant progeny. Certain of the stable *new donor* strains resemble the Hfr model more closely in that the donated fragments probably have one constant end, but again the recombinant progeny are donors rather than recipients.

In conclusion, we may say that the finding of a conjugation system, at least in part plasmid controlled, in a complex, differentiated, gram-positive bacterium, *S. coelicolor*, is of great interest in indicating the widespread importance of plasmids as genetic determinants in bacteria. These studies have added the streptomycetes to the gram-negative enteric bacteria, pseudomonads, and their relatives and the gram-positive staphylococci as examples of plasmid-carrying bacteria, although it should perhaps be noted that to date there is only genetic evidence for the existence of a plasmid in *S. coelicolor* (or any other streptomycete). There is, however, no reason to suppose that physical or biochemical evidence, or both, will not be forthcoming. Additional work will be required to elucidate the morphological basis of conjugation in *S. coelicolor*. Sermonti, Puglia, and Ficarra (92) have attempted to analyze the mating process in NF × UF crosses by blending the mixed cultures after different periods of incubation

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and determining the relative proportions of various classes of recombinant progeny among the plating units obtained. They found evidence that the proportion of recombinants inheriting donor markers some distance from the 9 o'clock region tended to increase with time, but whether this finding is to be interpreted as indicating a basic similarity between the mating and genetic transfer processes of *S. coelicolor* and *E. coli* remains to be seen.

ANALYSIS OF DIFFERENTIATION

Phases of Colonial Development

The mature colony of *S. coelicolor* on agar media is in effect a multicellular, differentiated organism comprising: the substrate mycelium, which has a purely vegetative function, growing on and into the medium; the aerial mycelium, whose function is the production of spores; and the spores themselves, which represent the distributive phase of the organism and are relatively resistant to desiccation (47, 109). After spore germination, the early growth of the colony is entirely vegetative, and young colonies are translucent and "bald" (i.e., devoid of aerial mycelium). The formation of aerial branches is macroscopically visible as an opaque, chalky layer on the surface of the colony, which then turns grey as the aerial hyphae form spore chains.

Ultrastructural studies show that aerial hyphal cells, unlike those of the substrate mycelium, possess a distinctive superficial layer, the "fibrous sheath" (55, 113), the function of which is not known, but which persists even on detached mature spores. Comparable superficial layers are also found on many other streptomycetes (110, 111, 115).

Aerial hyphae form spore chains through a sequence of events involving coiling of the hyphal tip, subdivision of the long apical cell by the simultaneous, double annular ingrowth of a number of closely spaced sporulation septa, and rounding up of the newly formed spore units accompanied by thickening of the spore wall (112; A. C. McVittie, manuscript submitted for publication). Spores generally contain a single gene complement. It should be emphasized that the structure and mode of formation of these spores is completely different from that involved in endospore formation in, for example, *Bacillus*, *Clostridium* (84), *Thermoactinomyces* (23, 24, 32, 75), and other bacteria of doubtful relationship (93).

Thus, *S. coelicolor* has macroscopic features which facilitate the detection of morphological mutants, microscopic features to help in understanding the nature of mutational blocks, and an excellent recombination system permitting genetic analysis of mutants. These facilities have been exploited in studies of white (*whi*) and, to a lesser extent, bald (*bld*) colony mutants (17, 18, 60; A. C. McVittie, manuscript submitted for publication).

Characterization of Morphological Mutants

Phase contrast microscope observation of the aerial mycelium of more than 50 *whi* mutants has led to the identification of six broad phenotypic classes (Fig. 10) ranging from phenotype I, in which the aerial hyphae are "fibrous" and show neither helical coilings nor any sign of spore formation, to phenotype VI, in which numerous spores sometimes indistinguishable from wild-type spores by presently available criteria, are formed (17; A. C. McVittie, manuscript submitted for publication). Thus the use of *whi* mutants will help to identify a sequence of events in sporulation, each of which occurs only upon accomplishment of earlier events. It is, however, possible that there are aspects of sporulation that are not dependent upon this sequence and which will not, therefore, be detected by the use of *whi* mutants. Alternative methods of isolating morphogenetic mutants would be very desirable.

Genetic mapping of the *whi* mutations has so far revealed nine distinct map locations (Fig. 2) (17, 60). Several mutations have been found at many of these locations, probably indicating that few loci remain to be identified. Mutations at each locus generally result in the same phenotype. (An exception to this is *whi-99* (phenotype V), which is located very close to the *whiG* locus (phenotype I) and which may represent a 10th locus, *whiF* (17)). Thus, it is generally possible to select representative mutants of each locus for further study. In the absence of meaningful complementation and dominance tests, which have not yet been devised for *whi* mutants, the possibility that some *whi* loci are gene clusters has not been eliminated, although the frequencies of recombination between mutations mapping together have suggested that *whiA*, *E*, *G*, *H*, and *I* are single genes (17 and K. F. Chater, unpublished data).

Knowing the map locations and phenotypes of various *whi* mutations, we can attempt to answer the question of the sequence of gene expression (where the phenotypes leave this in doubt) by examining the phenotypes of appropriate double *whi* mutants. To date, *whiG*

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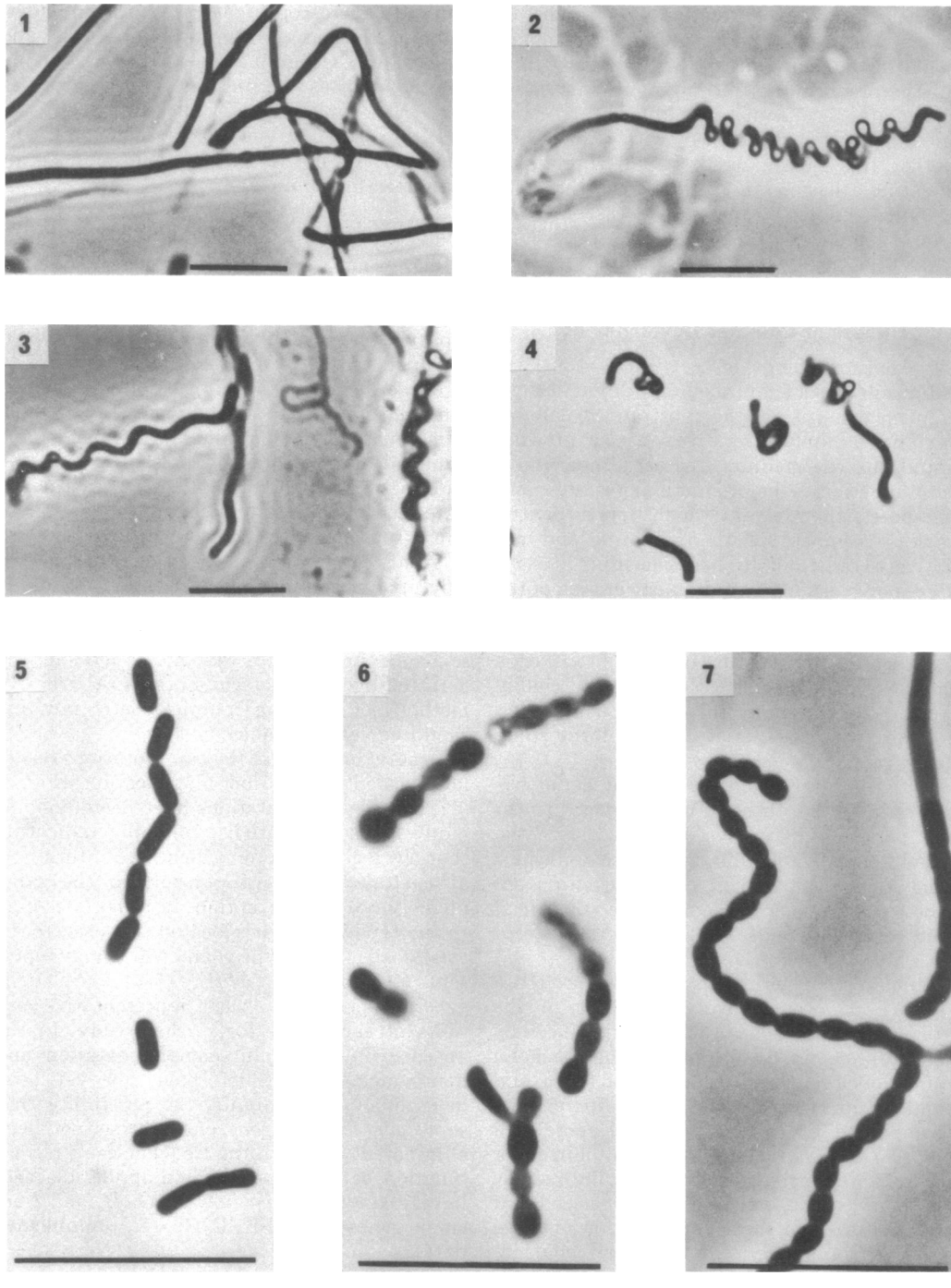

FIG. 10. *Phase-contrast photomicrographs of aerial growth of whi mutants. (1) whiG, phenotype I; (2) whiA, phenotype II; (3) whiH, phenotype III; (4) whiI, phenotype IV; (5) whiF, phenotype V; (6) whiD, phenotype VI (in this example the spores are abnormally spherical due to a defect in spore wall synthesis); (7) wild-type. Bar markers indicate 10 μm.*

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(phenotype I) and *H* (phenotype III) have been found to be epistatic to *whiA* and *B* (both phenotype II) and to *whiI* (phenotype IV), and *whiG* is almost certainly epistatic to *whiH* (K. F. Chater, unpublished data). Morphological studies (17, 60; A. C. McVittie, manuscript submitted for publication) have shown that *whiA*, *B*, *G*, and *H* mutants are all unable to make sporulation septa, while in *whiI* mutants these septa are abnormally spaced; the single *whiD* mutant has thin-walled spores; the single *whiF* mutant makes spores that fail to round up; and *whiE* mutants make morphologically apparently wild-type spores. Combining the genetic and morphological information, the sequence of events given in Fig. 11 is indicated. The place in this scheme of two mutants, *whiC193* and *whi-53*, which are both type I, but oligosporogenous (17; A. C. McVittie, manuscript submitted for publication), is uncertain.

Only a small number of *bld* mutants (Fig. 12) have been isolated; one of these (*bldA1*) is located between *ilvA* and *pel*, apparently near to the *whiB* locus, whereas others have been located, more approximately, in the 1, 6, and 9 o'clock regions (D. A. Hopwood, K. F. Chater, and E. N. Lawson, unpublished data).

Biochemical and Physiological Approaches to the Study of Differentiation in S. coelicolor

Clearly, there must be chemical signals involved in triggering key events in differentiation (particularly in aerial mycelium formation), and these signals must be transmitted to the transcription and/or translation machinery of the cell. We have so far only begun attempts to analyze the role of transcriptional control in differentiation, and our efforts have been confined to preliminary in vitro studies of deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase and to the isolation of rifampin-resistant mutants. Three resistance loci have been mapped, and mutations in only one (*rifA*) appear to decrease the sensitivity of RNA polymerase to rifampin in crude extracts (K. F. Chater, manuscript submitted for publication). At this stage, none of about 100 *rifA* mutants isolated has been found to possess morphological abnormalities attributable to the *rifA* mutation. This contrasts with the situation in *B. subtilis*, where Sonenshein and Losick (94) and Doi et al. (31) had no difficulty in finding rifampin resistant mutants altered in sporulation.

Our studies of the enzyme itself have not yet proceeded beyond preliminary characterization, but several interesting features of the enzyme, not connected with differentiation, have emerged (K. F. Chater, manuscript submitted for publication and unpublished data). Like that of *Bacillus subtilis*, the enzyme is highly sensitive to KCl; it behaves like that of other bacteria (e.g., *E. coli;* ref. 10) in its sedimentation characteristics in low and high salt glycerol gradients; it is scarecely inhibited at all by rifampin at 1 μg/ml and only about 70% at 20 μg/ml, in contrast to the high sensitivity of the enzymes of all other bacteria examined (104); and it is unusually sensitive to streptolydigin (50% inhibition at about 0.75 μg/ml) compared with other bacterial RNA polymerases (e.g., *E. coli* where about 5 μg/ml gives 50% inhibition; ref. 14). We have found that the enzyme of *Streptomyces albus* strain CMI 52766, on the other hand, is sensitive in crude extracts to rifampin concentrations as low as 0.01 μg/ml, but is also highly sensitive to streptolydigin (K. F. Chater, manuscript submitted for publication).

A particularly interesting possibility opened up by advances in streptomyces phage techniques (see below) is that phages may be found whose DNA is transcribed only by RNA polymerase from vegetative hyphae, and not by that from sporulating hyphae. This would provide an elegant test of RNA polymerase template specificity analogous to that described by Losick and Sonenshein (71) for *B. subtilis*, where vegetative, but not sporulation, RNA polymerase transcribes phage φe DNA. At present, this approach is hampered by the difficulty of obtaining either phage DNA or sporulating hyphae in useful quantities.

GENETIC ASPECTS OF PHAGES

Isolation and Characterization of Phages

Although bacteriophages attacking streptomycetes have been known for more than 35 years (107, 108), it is only very recently that phages for *S. coelicolor* A3(2) have been isolated. They have come from two sources.

Dowding (J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972) isolated 28 phages from soil samples either by plating membrane filtrates of soil suspensions directly in top layers with the host or after overnight enrichment of the soil sample by incubation in nutrient broth with host spores, followed by membrane filtration and plating as before. The specific enrichment method proved more reliable, resulting in the isolation of

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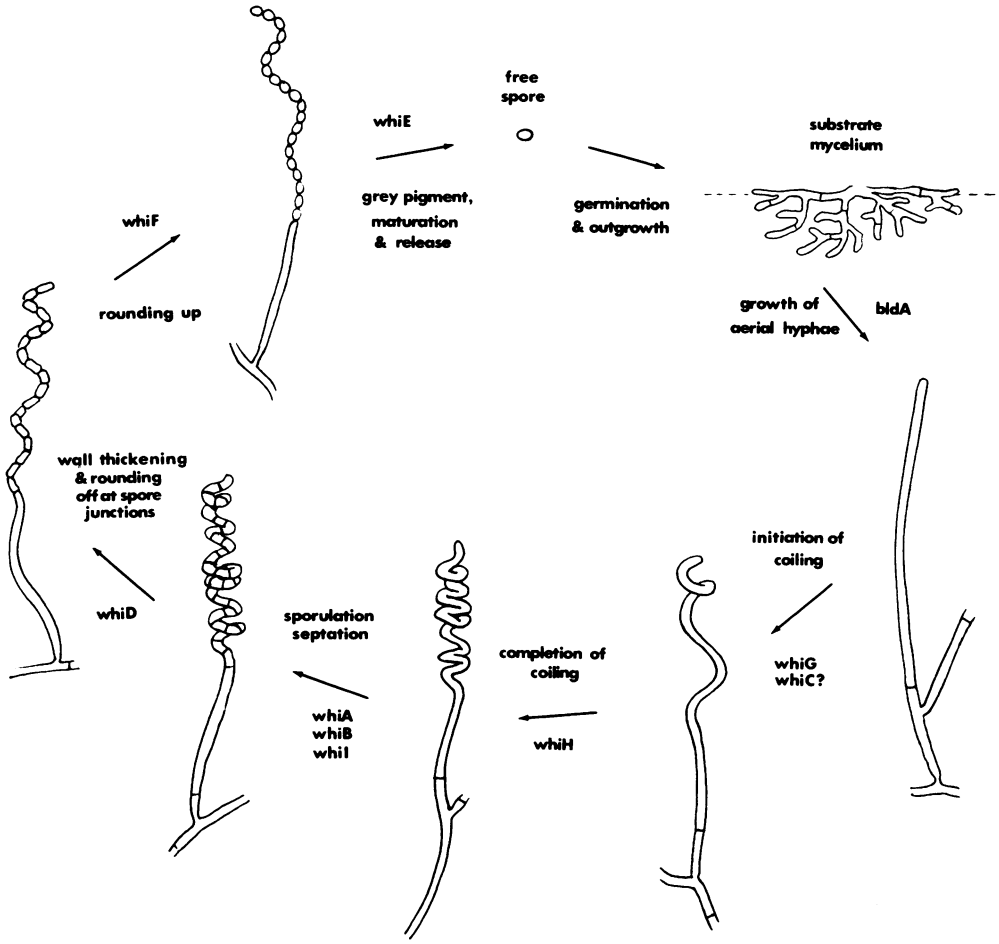

FIG. 11. *Diagrammatic representation of the genetic control of morphogenesis in S. coelicolor. The designations of genes thought to be implicated in each step are given outside the arrows. The diagram incorporates information obtained from genetic mapping tests of epistasis and light and electron microscopic studies (17, 60; A. C. McVittie, manuscript submitted for publication; K. F. Chater, unpublished data).*

phages from 25 out of 28 soil samples. The plaque morphologies of this group of phages fell into four types (Fig. 13). The group of phages with type IV plaques may perhaps be heterogeneous, the small size of the plaques simply reflecting the fact that optimal conditions for plaque development by these phages have not yet been defined. A representative phage with type III plaque morphology (large, clear plaques) is the virulent phage VP11 (33), whereas the temperate phage VP5 (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press) is an example of a phage with type II plaque morphology, the plaques having a turbid margin. During prolonged incubation the plaques of VP5 increase in size and come to contain concentric rings of surviving and lysed host (Fig. 14), an appearance that has been interpreted in terms of changes in the multiplicity of infection and consequently in the relative probabilities of the lytic cycle and of lysogeny (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press).

The second source of a phage for strain A3(2) is the strain itself. Lomovskaya et al. (68) showed that A3(2) carries a defective prophage, but nevertheless releases phage at a very low frequency, presumably as a result of mutation of the prophage to a nondefective form, followed by spontaneous induction. The phage so pro-

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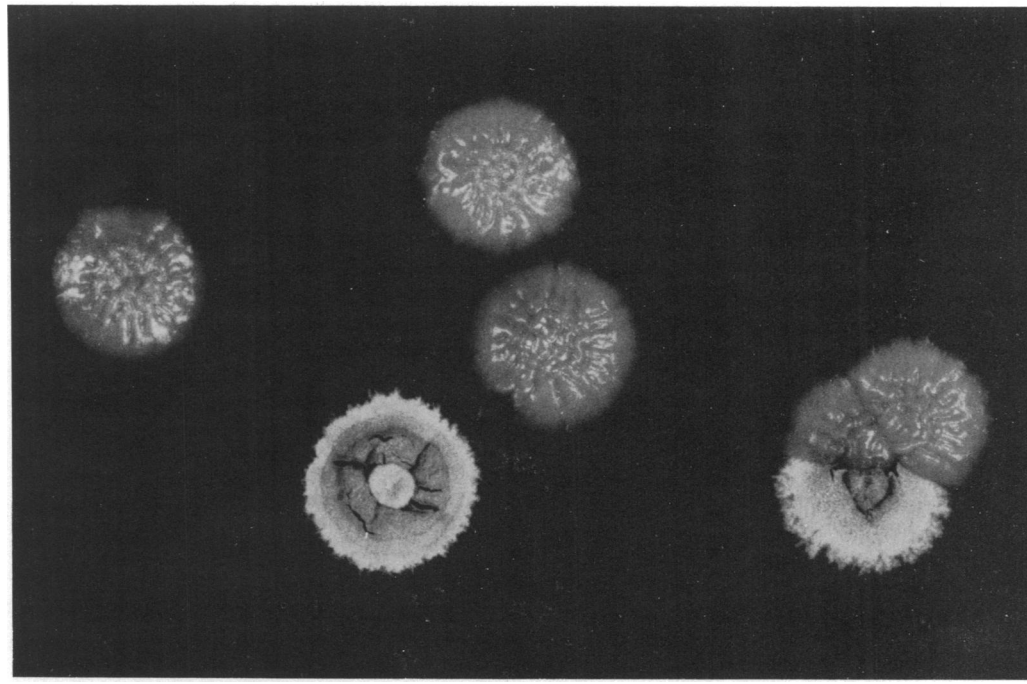

FIG. 12. *Colonies of wild-type and bld (bald) colony mutants; the latter are distinguished by their smooth, shiny appearance.* (×5.5).

duced, ϕC31, lysogenized an indicator strain and also A3(2), which had been cured of the defective prophage by UV irradiation.

Dowding (J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972) examined six of his phages by negative staining in the electron microscope and found that they all had long, striated, flexible tails, thus belonging to group B of Bradley's morphological classification (8). The virulent phage VP11 and the temperate phage VP5 had slightly different head and tail dimensions (33; J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press) (Fig. 15). No icosahedral RNA phages or other sex-specific phages have yet been described for *S. coelicolor* or any other streptomycete, and it may be that they do not exist. Those associated with *E. coli* (22), *Pseudomonas* (9), and *Caulobacter* (85) have all been shown to adsorb to pili, and so far these have not been demonstrated in *Streptomyces*.

Phage Mutants and Recombination

Lomovskaya et al. (69) isolated clear-plaque mutants of the temperate phage ϕC31, as well as mutants able to grow at 37 C, a temperature too high for reproduction of the wild-type phage. By mixedly infecting the host with phages differing in these two characteristics, it was demonstrated that phage recombination occurred.

Clear-plaque mutants of phage VP5 were also isolated (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press), spontaneously or after mutagenesis of free phage by UV or hydroxylamine; they have been used in a search for nonsense suppressors (V. Najfeld, personal communication) but so far not in recombination experiments. In the virulent phage VP11, Dowding (33) obtained a high yield of temperature-sensitive mutants and plaque morphology mutants after mutagenesis of intracellular phage by NTG (97) and used them to demonstrate phage recombination. A reproducible value of 5 to 6% wild-type recombinants was obtained in crosses between a pair of temperature-sensitive mutants.

Lysogeny

Probably because of their mycelial growth habit, which results in a culture containing cells of diverse morphological and physiological state, some unfavorable for phage multiplication, streptomycetes are liable to show the

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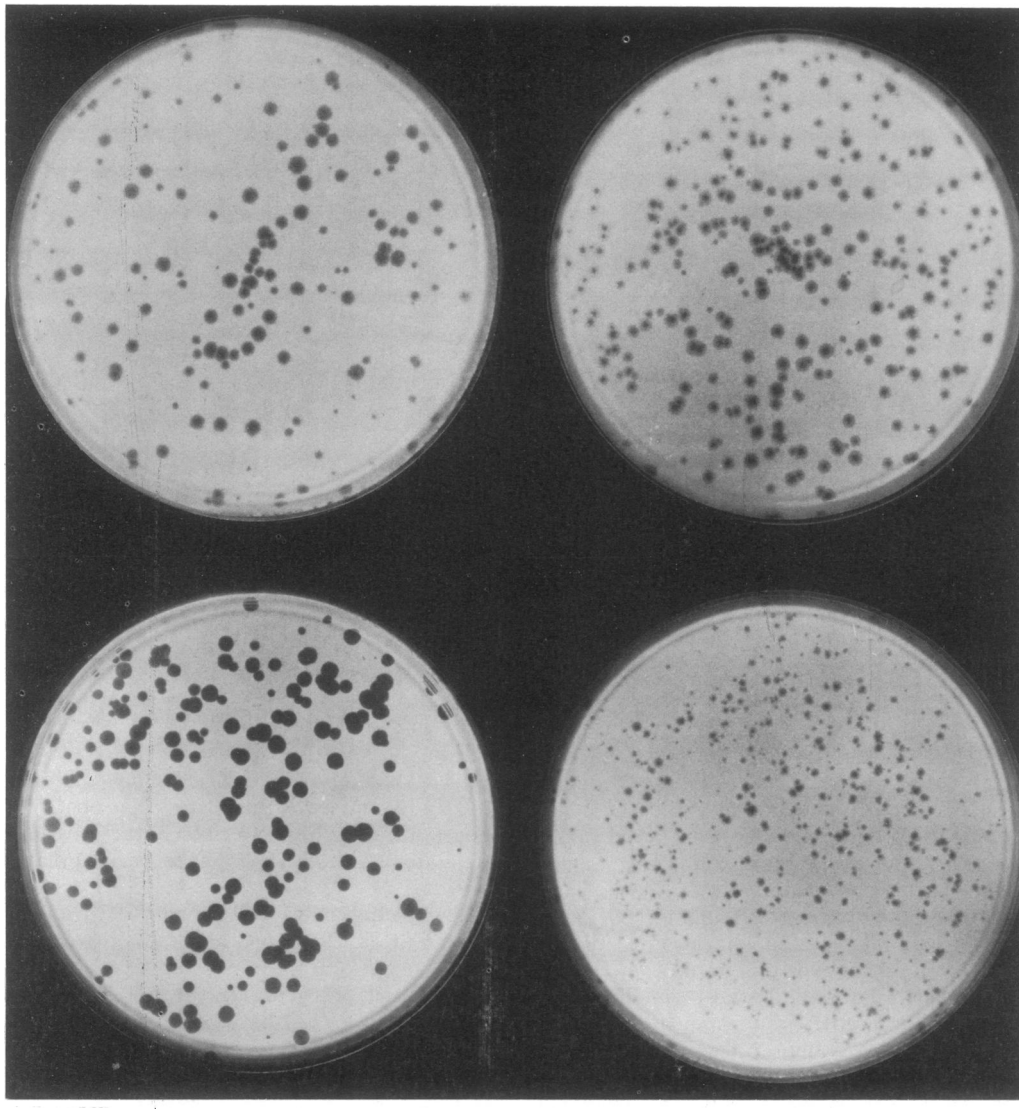

FIG. 13. *Four plaque types among phages capable of growth on S. coelicolor A3(2). I, VP9; II, VP5; III, VP11; IV, VP19. (From J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972.)*

phenomenon of "pseudolysogeny" (106). A virulent phage usually lyses only a part of a culture before phenotypically resistant cells arise, and these can be propagated indefinitely, along with the phage, superficially mimicking true lysogeny unless the culture is rigorously cloned, preferably under conditions unfavorable to phage adsorption, to free it from trivially carried phage.

True lysogeny has, however, been demonstrated in several streptomycetes (69, 105, 106; J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press); the temperate phages involved, with the exception of VP5 and two other closely related phages, were derived from a lysogenic culture shown to carry a prophage by the production of plaques on a sensitive indicator strain.

The two phages ϕC31 and VP5 are heteroimmune as shown by the fact that A3(2) is sensitive to VP5, but resistant to ϕC31, unless cured of the defective ϕC31 prophage, and by the finding that an indicator strain sensitive to ϕC31 is still sensitive to it when lysogenized by VP5 (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press). Mapping experi-

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ments, in which defective lysogens of ϕC31 (68) or VP5 (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol, in press) were crossed with nonlysogenic strains, have revealed a chromosomal attachment site for each prophage (Fig. 2). They turn out to have interesting locations, because certainly VP5, and probably ϕC31, integrate in the long "silent region" of the linkage map (50) which contains no normal markers, raising the possibility that the genetic length of this region may in part be due to enhanced crossing over through some kind of site-specific recombination involving prophage attachment sites (J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press).

Transduction

There is still no unambiguous demonstration of transduction in a streptomycete; the first report of transduction in *S. olivaceus* (3) has not led to a reproducible system, and preliminary attempts to demonstrate transduction by VP5 in *S. coelicolor* A3(2) gave negative results (J. E. Dowding, Ph.D. thesis, Univ. of East Anglia, Norwich, England, 1972). However, now that several temperate phages for this strain have been isolated, there is a good chance that transduction will become a reality.

RADIOBIOLOGY

Ultraviolet-Sensitive Mutants

Ninety-four ultraviolet-sensitive mutants, isolated by a simple replica-plating technique, have served to identify six loci concerned with the repair of UV damage in *S. coelicolor* A3(2) (41–43). Three of these, *uvsC*, *A*, and *D*, are closely clustered, two others, *uvsB* and *E*, are diametrically situated with respect to the first cluster, and the sixth, *uvsF*, is not closely linked to either of the two clusters.

The functions of these loci have not been defined. However, mutations in some of them could be differentiated phenotypically. *uvsA*, *B*, *C*, and *D* mutants had survival curves with a shoulder and an exponential region as in the wild type, but showing a sensitivity increased by a factor of 7 to 20; the two *uvsB* mutations appeared to confer somewhat less sensitivity than most *uvsA*, *C*, and *D* mutations. Mutations in *uvsE* differed from the others in abolishing the shoulder of the wild-type curve but retaining a similar slope in the exponential part

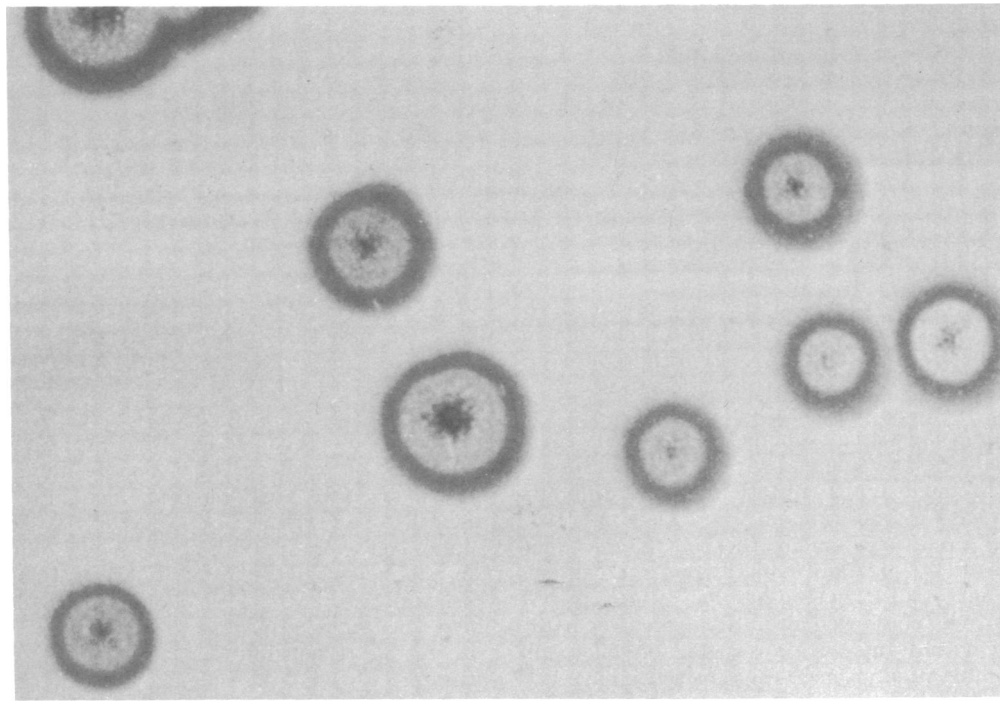

FIG. 14. *Three-day-old plaques of the temperate phage VP5 on S. coelicolor A3(2) showing a clear, lysed center and ring of lysogenised surviving growth surrounded by a further ring of lysis.* (×5).

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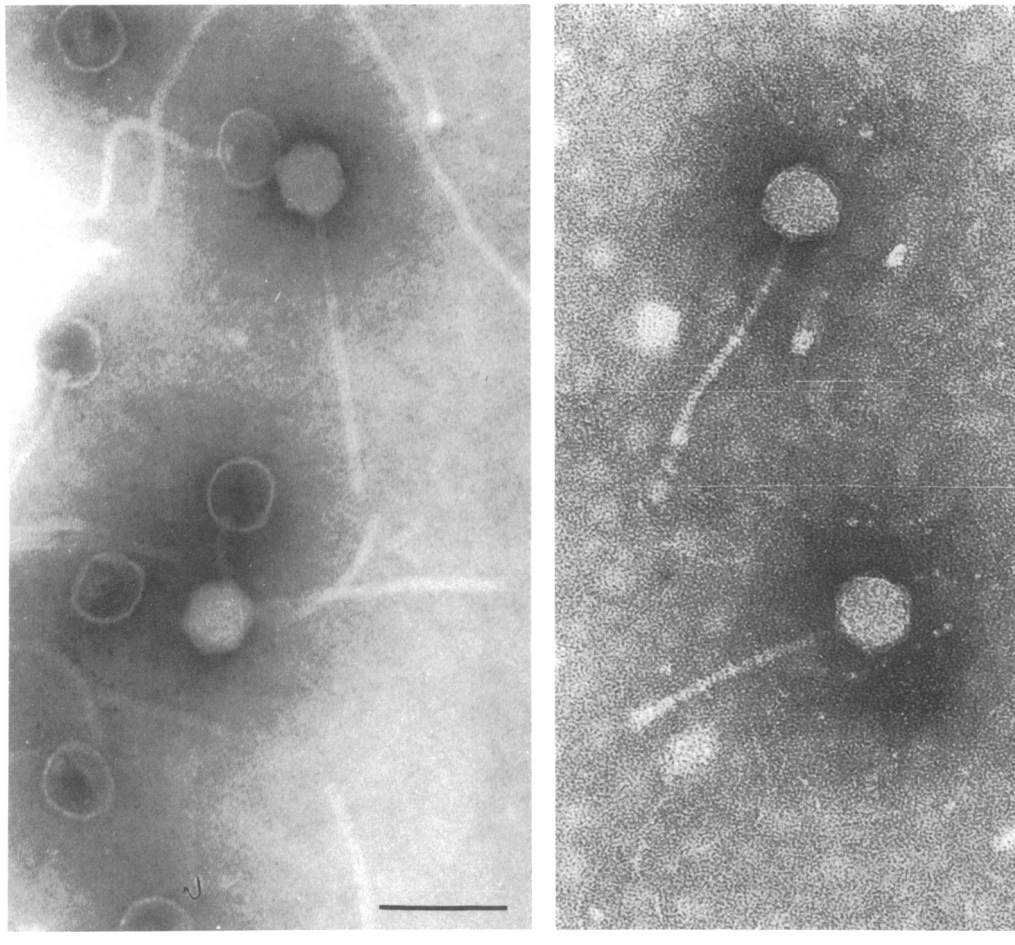

FIG. 15. *Electron micrographs of virulent phage VP11 (left) and temperate phage VP5 (right) negatively stained with neutral potassium phosphotungstate. Marker bar represents 100 nm (from ref. 33; J. E. Dowding and D. A. Hopwood, J. Gen. Microbiol., in press).*

of the curve, which in the case of the *uvsE* mutants began from the origin. The single mutation characterizing *uvsF* resulted in no increase of UV sensitivity unless combined with a *uvsA*, *C*, or *D* mutation, when the double mutant had a greatly increased sensitivity compared with the single *uvsA*, *C*, or *D* mutants.

The incidence of mutations in the different loci was far from random; there were only two *uvsB* mutations among the first 23 mutants isolated (41, 42) and none among the next 71 mutants (43). Thus, other loci affecting UV sensitivity could well await discovery. In particular, no mutants with a recognizable effect on recombination or on X-ray sensitivity have yet been found.

It seems likely, particularly in view of the finding that the *uvsA*, *C*, and *D* loci control host cell reactivation of UV-irradiated phage VP11 (33), that these three genes are concerned with the excision repair well characterized in other bacteria and would therefore be the counterparts of the *uvr* loci of *E. coli* K-12 (62). Harold and Hopwood (41) concluded, from a consideration of the survival curves of double mutants (*uvsA uvsB*, *uvsC uvsB*, *uvsD uvsB*), that *uvsB* might also be concerned with part of the same system. However, the finding that a *uvsB* mutation had no effect on host-cell reactivation of UV-irradiated phage VP11 (33) makes this less likely. In formal terms, the *uvsE* locus can be said to be responsible for a repair system saturable at low UV doses, hence the lack of the shoulder in the survival curves of *uvsE* mutants, whereas the *uvsF* locus can be postulated to be capable of repairing a proportion of the damage

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also reparable by the *uvsA*, *C*, and *D* system (41); this locus also had no detectable effect on repair of phage VP11, even in the presence of a *uvsD* mutation (33).

Photoreactivation

Photoreactivation was first discovered in *Streptomyces griseus* (65). Hence, it came as a surprise that *S. coelicolor* A3(2) is incapable of enzymatic photoreactivation (41). This is not a characteristic of all strains of *S. coelicolor*, however, some of which show a classical photoreactivation with a dose reduction factor of about 0.5 (41).

ANALYSIS OF BIOSYNTHETIC AND DISSIMILATORY PATHWAYS

What little is known of biosynthetic pathways in *S. coelicolor* depends almost wholly on the responses of biochemical mutants to biosynthetic intermediates and on genetic mapping and complementation. Specific studies of biosynthetic pathways have been initiated for histidine, tryptophan, and the aromatic amino acids.

Tryptophan Synthesis

Engel (34 and personal communication) has studied 28 tryptophan auxotrophs (*trp*) and has recognized at least four loci on the basis of phenotypes, including enzyme defects (Table 2), and genetic mapping. Two clusters of genes are indicated, *trpA* and *B* mapping between *hisC* and *ammA*, and *trpC* and *D* mapping between *rifB* and *thiC*. This situation contrasts with that prevailing in *E. coli* (116), *Salmonella typhimurium* (8), and *B. subtilis* (13), where the *trp* genes are arranged in a single contiguous cluster, and perhaps resembles that in *Pseudomona putida*, where *trp* structural genes map in at least three regions (40). No *trp* mutants of *S. coelicolor* have been isolated that respond to anthranilic acid, although Watkins (C. A. Watkins, M.Sc. thesis, Virginia Polytechnic Institute and State University, 1972) observed that the tryptophan requirement of aromatic amino acid auxotrophs was satisfied by this compound.

Aromatic Amino Acid Synthesis

The pathway of aromatic amino acid biosynthesis in cell-free extracts of a strain of *S. coelicolor* different from A3(2) resembled that in other bacteria (6), although there is evidence that in several streptomycetes, the first enzyme is unusual in being feedback inhibited only by tryptophan (38, 64). Watkins (C. A. Watkins, M. Sc. thesis, Virginia Polytechnic Institute and State University, 1972) studied five mutants (*aro*) having multiple aromatic amino acid requirements. One *aro* mutation was located between *proA* and *hisC*, defining the *aroB* gene. The *aroB* mutant required tryptophan plus tyrosine plus phenylalanine plus *p*-aminobenzoic acid. The other four *aro* mutations, which had less extensive requirements, were located between *argA* and *hisE* and formed two complementation groups defining the *aroA* and *C* genes. None of the mutants responded to shikimic acid.

Histidine Synthesis

The major recent advance reported in this research has been the detection (12) of a *cis*-dominant deletion mutation of part of the major *his* gene cluster, which causes constitutive synthesis of at least two enzymes specified by genes of the cluster, but does not affect expression of the *hisD* gene, which is located far from the cluster (81).

Dissimilatory Pathways

Dissimilation in *S. coelicolor* has been almost completely ignored. Recently, however, Sabater, Sebastian, and Asencio (82, 83) have characterized constitutive glucokinase and inducible mannokinase and fructokinase activities in an independently isolated strain of *S. violaceoruber*. It would be of great interest to repeat these results in *S. coelicolor* A3(2), in which a genetical approach could also be employed, particularly now that agar nonutilizing (*aga*) mutants are available, from which the isolation of strains defective in the use of various carbon sources has been simple (K. F. Chater and F. Flury, unpublished data).

Carbon Dioxide Mutants

The occurrence of mutants of various microorganisms auxotrophic for carbon dioxide has been reviewed by Charles and Broadbent (15) and Charles and Roberts (16). In most cases the mutants respond to particular alternative nutrients, sometimes already explicable in terms of known biosynthetic pathways, and most of the carbon dioxide mutants of *S. coelicolor* isolated by Vivian and Charles (101) were of this type. However, one mutant, defining a locus *cdxA*, appeared to have a carbon dioxide requirement for which no alternative nutrient was found. In view of the possible role of carbon dioxide as an environmental factor influencing colonial development in microorganisms (5, 70), a deeper study of the response of mutant and wild-type

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S. coelicolor to carbon dioxide appears to be worthwhile.

CONCLUDING REMARKS

From this review it is apparent that, in spite of progress in a number of directions, there are major areas where little or no research has been done and which could profitably be explored. One is in biochemical genetics, where the time is surely ripe for thorough investigations of biochemical pathways. Already there is evidence of different groupings in *S. coelicolor* of genes concerned with particular pathways as compared with other bacteria. Regulation of gene expression and enzyme activity in a differentiated soil bacterium would undoubtedly provide informative comparisons with enteric bacteria, pseudomonads, and bacilli. Some mutants are already mapped and available in many biosynthetic pathways, and a similar situation for catabolic pathways will soon exist.

A second general area where research is needed is in central aspects of molecular biology, such as DNA replication, transcription, and translation. Such research would obviously complement the genetic studies on phages, plasmids, and differentiation which are being actively pursued in our laboratory. The integrated picture of the molecular biology, biochemistry, and genetics of *S. coelicolor* that would emerge from these studies would not only be valuable in terms of comparative biology, but also in the context of the large-scale production of antibiotics and other substances by streptomycetes; the biosynthesis of antibiotics (21, 29, 74, 99) and its regulation could be studied more incisively, and methods of transferring the genetic determinants for high yield into other strains or even species having superior cultural characteristics might well be developed through the agency of plasmids or phages. It is significant that genetic studies of actinomycetes other than *S. coelicolor* are proliferating (52) and that much more interest in recombination studies is currently being shown by industrial laboratories, a sure sign that some successful applications of recombination in strain improvement are anticipated.

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