**REVIEW
ARTICLE**

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Forty years of genetics with *Streptomyces*: from *in vivo* through *in vitro* to *in silico*

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Beginnings: the A3(2) strain

As an undergraduate at Cambridge in the early 1950s, I had found genetics the most exciting part of my courses. This was the main motivation for choosing Botany as my Part II (final year) option in the academic year 1953/54. Granted, there was the Department of Genetics at Cambridge, headed by none other than Ronald Fisher, the father of biological statistics and a true genius, but an advanced qualification in mathematics was a *de facto* entry ticket to Part II Genetics, reflecting the strong mathematical basis of research in that Department. In the Botany School, 'biochemical genetics' was being studied – using *Neurospora crassa* – by Harold Whitehouse and Lewis Frost. During the Part II course in Botany, they far-sightedly emphasized the power and promise of microbial genetics for the future understanding of gene structure and function. In choosing a PhD project to start in October 1954 I heeded their advice. One of the topics on offer was *Streptomyces*. Here was a group of microbes apparently 'intermediate between bacteria and fungi', and so they could not fail to be interesting from a genetic point of view, given the clear differences that were emerging between the genetics of the few bacteria so far studied and the genetics of fungi and higher organisms. In the Pneumococcus, *Escherichia coli* and *Salmonella typhimurium*, incomplete genomes were transferred from donor to recipient strains, by any of three bizarre processes (transformation, conjugation and transduction), to yield incomplete zygotes, whereas in fungi and higher organisms (except those that were asexual) life cycles, including a complete diploid stage and meiosis, were the rule.

To get me started, Harold Whitehouse lent me a copy of Waksman's book on the actinomycetes (Waksman, 1950), newly purchased from Heffers' bookshop, and Lewis Frost handed over half a dozen cultures of *Streptomyces* that he had obtained from his friend George Floodgate at the then Royal College of Technology in Glasgow (now Strathclyde University). I streaked them out: several grew well, and one produced a striking blue pigment. I could therefore (I thought!) name it *Streptomyces coelicolor*. As a bonus, pigmen-

tation might in due course make a valuable genetic marker, as it had in *Aspergillus nidulans*. I chose this culture (strain 204F) and set about isolating auxotrophic mutants in order to look for genetic recombination *à la* Lederberg & Tatum (1946). Pretty soon, I could grow pairs of mutants together and select rare prototrophs from the progeny spores. In crosses of two doubly marked strains, non-selected markers from each parent segregated amongst the progeny, indicating a genuine process of gene reassortment rather than simply some kind of heterozygote formation. However, by early 1955 problems had arisen: the cultures started to grow erratically and became 'friable' (Fig. 1). I diagnosed phage infection, but could not cure it. I therefore wrote to Dagny Erikson, at the University of Aberdeen, to ask for cultures of *S. coelicolor*. She had published extensively on variation in cultures that she had derived from Stanier's agar-liquefying strain (Stanier, 1942), including some derived by using a micromanipulator to isolate individual spores from the same chain (Erikson, 1948). Her initial response was disappointing: she had retired to Kincardineshire to raise a family (as the new Mrs A. E. Oxford) and doubted whether any viable cultures remained. However, she would see if Dr D. M. Webley could help. A week later, a further letter arrived: 'Dr Webley informs me that his culture of *Streptomyces coelicolor* is not in good condition. I have found an old sterile soil ampoule which was sown with the blue-pigmented, agar-liquefying strain A3(2). I cannot guarantee it, but hope it may still be viable and pure.' Indeed it was! I shook some soil particles from the ampoule onto an agar plate and beautiful blue colonies grew out. I made auxotrophic mutants and they were stable, and grew perfectly. They produced prototrophic recombinants (Fig. 2) when grown in pairs (Hopwood, 1957). Thus was the A3(2) strain launched on its genetic career. Meanwhile, the true phylogenetic relationships of *Streptomyces* needed looking into.

Streptomycetes are bacteria

Early in the development of microbiology, there were several descriptions of novel organisms that are now included in the actinomycetes, to which *Streptomyces*

Fig. 1. Colonies of *S. coelicolor* strain 204F: left, normal sporulating colonies of the original strain; right, a 'friable', asporogenous culture, possibly caused by phage infection. Reproduced from Hopwood (1958).

Fig. 2. Some of the first recombinants selected from a four-factor cross between marked derivatives of *S. coelicolor* A3(2), *metA2* × *hisA1 argA1 strA1*. Left, recombinants on a medium containing histidine (selection for *met⁺/arg⁺*); right, recombinants on a medium containing arginine, methionine and streptomycin (selecting *his⁺/str*). Note the slight background of parental growth on the left-hand plate caused by cross-feeding of the parental strains and its absence on the right-hand plate because one of the parents is killed by the streptomycin. Reproduced from Hopwood (1958).

belongs. Hansen (1874) saw the causal agent of leprosy in tissue samples from human patients and called it *Bacillus leprae*; Cohn (1875) named as *Streptothrix foersteri* a filamentous organism from human tear ducts; and Harz (1877) described *Actinomyces bovis* in the lesions of 'lumpy jaw' in cattle. It was probably the naming of this last organism as 'ray fungus', because of the tendency of the elongated, branching cells to grow radially from the centre of the lesion, that later contributed to the confusion surrounding the taxonomic position of the *Actinomycetales*; the renaming of Hansen's bacillus as *Mycobacterium* ('fungus rodlet') and Cohn's *Streptothrix* ('twisted hair') as *Streptomyces* ('twisted- or chain-like fungus') did not help.

By the early 1950s, few microbiologists regarded the actinomycetes as fungi, but many still thought of them as intermediate between fungi and bacteria. For *Strepto-*

myces, there were biochemical pointers to a bacterial affinity – the cell-wall composition resembled that of typical Gram-positive bacteria, and they were sensitive to specifically anti-bacterial antibiotics – but their cellular architecture was unclear. In Cambridge, through a mutual friend (Martin Canney), I was lucky enough to meet the brilliant electron microscopist Audrey Glauert who, with Ernst Brieger, was studying the fine structure of *Mycobacterium* strains at the Strangeways Laboratory. This was the start of a long and rewarding collaboration during which we showed, among other things, that *S. coelicolor* lacks a nuclear membrane (Fig. 3) and so is, by definition, a bacterium (or 'prokaryote' to use a later term). Nevertheless, it was clearly very different morphologically from the simple, rod-shaped bacteria that had hitherto been studied genetically, so the idea that it might reveal novel genetic phenomena was still very much alive.

Fig. 3. A thin section of a germinated spore (S) of *S. coelicolor* A3(2) with its germ tube, together with two other spores, showing prokaryotic cellular architecture. The hypha is bounded by the cell wall (W), under which lies the plasma membrane (PM), which is continuous with peripheral pockets of membranous material (P). The fibrillar nuclear material (N) extends from the spore into the germ tube, and from the hypha into a side branch and is not delimited from the cytoplasm by a membrane. Intracytoplasmic membranous regions (CM) – later called mesosomes – are also seen. There is an irregular layer of material (M) on the outer surface of the wall. See also Glauert & Hopwood (1961).

Does *Streptomyces* have a life cycle?

The very first issue of the *Journal of General Microbiology* (forerunner of *Microbiology*) contained two papers by Dagny Erikson on 'Differentiation of the vegetative and sporogenous phases of the actinomycetes' Erikson (1947a,b), and also one by Emmy Klieneberger-Nobel on 'The life cycle of sporing *Actinomycetes* as revealed by a study of their structure and septation' (Klieneberger-Nobel, 1947). In the latter was described, for four strains of what we would now call *Streptomyces*, a life cycle consisting of two growth phases: a 'primary' or 'substratum' mycelium gave rise, by a process of cellular fusion (and implied fusion of 'chromosomes') in special hyphal aggregates, to 'initial cells'; these then produced the sporulating, 'secondary' or 'aerial' mycelium by a germination-like process (Fig. 4).

If true, such a life cycle, with an implied regular alternation of haploid and diploid phases, would obviously have had profound implications for *Streptomyces* genetics. However, it could not be substantiated. Erikson (1955) had found that aerial hyphae of the A3(2) strain arose as simple side-branches of vegetative hyphae anywhere over the colonies. I confirmed this (Fig. 5), and criticised the interpretation by Klieneberger-Nobel of her own observations, concluding that 'there appear to be no objective reasons for Klieneberger-Nobel's interpretation of those of her photographs which are claimed to show initial cells' (Hopwood, 1960). In hindsight, this was perhaps a rather impertinently expressed criticism of a senior microbiologist by a brash neophyte, but the point appeared to be in need of

Fig. 4. Klieneberger-Nobel's 'life cycle' for *Streptomyces*. No. 1 is a spore, which germinates (2-4) to produce the 'primary' mycelium. In 5-6 are 'nests' of hyphae which go on (7) to produce 'initial cells' (8). These 'germinate' to produce the 'secondary' mycelium (9-10). Pairs of 'chromosomes' (11-12) enter developing spores (13-14). My criticism was the lack of objective evidence for distinguishing the round bodies called 'initial cells' (8) from the spores (1); instead of showing initial cells giving rise to aerial mycelium, 8-10 could instead be showing spores regenerating *in situ* in the aerial mycelium, as was later shown to occur under appropriate conditions (e.g. Dowding, 1973). Reproduced from Klieneberger-Nobel (1947).

Fig. 5. Phase-contrast photomicrograph showing a spore (left) of *S. coelicolor* A3(2) which has germinated to produce a vegetative mycelium on which two aerial hyphae (growing upwards in the photograph), one already going on to sporulate, have arisen as simple side branches. Reproduced from Hopwood (1960).

emphasis, especially because the idea of initial cells as a stage in the life cycle was beginning to be assumed in the literature (e.g. McGregor, 1954; Dickenson & MacDonald, 1955).

Genetic studies in other laboratories

As is often the case in science, I was not alone in my work: the time was evidently ripe to look for genetic recombination in *Streptomyces*. It transpired that at least five other groups had started similar work at about the same time, all unbeknown to each other (Sermonti & Spada-Sermonti, 1955; Alikhanian & Mindlin, 1957; Bradley & Lederberg, 1956; Braendle & Szybalski, 1957; Saito, 1957). Remarkably, two of them had also independently chosen blue-pigmented strains of *S. coelicolor*, probably for the same reason as I had done. While the possible intermediate phylogenetic position of *Streptomyces* had been a motivation for several groups, as it had been for me, most also had a desire to develop rational breeding methods in the group of microbes that were beginning to establish themselves as the world's most prolific antibiotic producers (Szybalski, 1959); this had not been a factor in my own case because I knew next to nothing about antibiotics. I was forced to take an interest in them only later when the genetics of the blue pigment started to open up. Most of the 'competition' dropped out after about 1960, while I developed a fruitful collaboration with the group who had published first, Giuseppe Sermonti and his wife Isabella Spada-Sermonti in Rome. They soon adopted the A3(2) strain, with its linkage map and my growing collection of multiply marked mutants and recombinants (see later section). Eventually, our paths diverged and the Sermontis moved to other fields in the 1970s.

Taxonomy of the A3(2) culture

I have to 'blame' Sermonti for the opprobrium that we later attracted for persisting in referring to the A3(2) strain as '*S. coelicolor*'. In 1959, Kutzner & Waksman (1959) concluded that *Streptomyces* strains that make blue pigments represent two distinct taxonomic groups (even the pigments are chemically quite different), and that a strain described by Müller had priority for the name *S. coelicolor*; strains resembling A3(2) should be called '*S. violaceoruber*'. I was convinced by the argument, and Audrey Glauert and I started to use the new name in our cytological papers (e.g. Glauert & Hopwood, 1961; Hopwood & Glauert, 1961). However, Sermonti persuaded me that there was no guarantee the name would not be altered again and that we would confuse geneticists by changing horses in mid-stream.

Establishing a linkage map

Having concluded that *Streptomyces* are bacteria and, like other bacteria, show no objective evidence of a life cycle involving anything other than asexual reproduction, the task was to try to establish a system that could illuminate the genetics of *S. coelicolor* A3(2) purely by analysis of recombinants produced in pairwise mixtures of auxotrophic strains. In trying to do so, I was mindful of the problems that bedevil any deduction of the linkage relationships of bacterial genes when recombinants have to be selected from a huge excess of asexually produced progeny of the two parental groups. The consequence of this is that the frequencies of particular classes of recombinants in respect of the non-selected markers do not simply reflect the distance apart of these markers, which one is trying to establish, but also the positions of these genes with respect to the selected and counter-selected markers, which might lie between the non-selected markers, or outside of them, or one between and one outside. These problems had been dramatically illustrated in the paper by Lederberg *et al.* (1951), in which the linkage map of *E. coli* K-12 (though presumably not the chromosome itself) had to be represented as a branched structure, before an understanding of the donor and recipient roles of the parents in the cross allowed sense to be made of the linkage data.

My solution to the problem came to me out of the blue one afternoon when I was explaining my project to two German colleagues from St John's College over tea in the Botany School, one a linguist and the other an economist (thereby doing nothing to dispel the idea prevalent among non-scientists that science advances by the Eureka principle!). This was the four-on-four cross. In it two parents each have two markers, which can be either selected or non-selected. Recombinants are recovered from equal numbers of total progeny on four media, each making one of the four possible selections of one marker from each parent and leaving the second marker of that parent non-selected. Because there are

two non-selected markers, four recombinant genotypes can grow on each medium; their frequencies are determined by picking and classifying samples of the colonies. Some of the genotypes grow on more than one of the media, while others are unique to a single medium. Taking them all together, they include one or both members of each of the seven possible complementary pairs of recombinant genotypes, leaving only the parental classes (which are, of course, the same as the vast excess of asexual progeny) unmeasured. In this way *relative* linkage distances could be measured and used to construct a map.

The first version of the map (Hopwood, 1958) had just six loci, in two linkage groups, and this was soon extended to 15 loci, with the (temporary) appearance of a third linkage group represented by a single gene (Hopwood, 1959). With the discovery of heteroclones – colonies deriving from partially diploid plating units, each of which gave rise to a population of haploid recombinants that could be analysed non-selectively (Sermonti *et al.*, 1960; Hopwood *et al.*, 1963) – the map grew to 39 loci, still in two linkage groups (Hopwood, 1965a). Soon, the map was firmly enough established for a simpler system of analysis to be employed. Selection was made for recombination between just two known points on the chromosome with other markers unselected. In appropriate crosses, the pattern of marker selection in one linkage group influenced, in very specific ways, the segregation of markers in the other linkage group; the interpretation was a single, circular linkage group that incorporated the two previously separate groups of genes (Hopwood, 1965b).

The reason it had not been possible earlier to bridge the gaps between the two original linkage groups was that they were separated by long, very sparsely marked segments. These so-called ‘silent regions’ were devoid even of a general class of temperature-sensitive mutants, as described in a paper (Hopwood, 1966a) that acknowledged for the first time the input of Helen Ferguson (later Wright, still later Kieser), who has contributed so much to our 35 year collaboration. These regions remained for long a striking feature of the *S. coelicolor* genome and would be interpreted only some 30 years later (see later section) when Helen was able to map the chromosome physically (Kieser *et al.*, 1992).

As Frank Stahl (1967) had taught us, this map circularity did not necessarily mean that the chromosome itself was a circle: merozygosity, leading to the requirement for even-numbered crossovers to generate recombinants between a complete chromosome from one parent and a fragment from the other (except in the rare case when the fragment included a chromosome end), could have been a sufficient explanation for circularity of the genetic map even if the chromosome were linear (Fig. 6). For technical reasons, it was not possible to obtain the kind of direct physical evidence that had established chromosome circularity in *E. coli* (Cairns, 1963), so I tried at least to distinguish between linearity on the one hand and circularity or circular permutation on the other by analysing several hundred heteroclones selected from

Fig. 6. Diagram to illustrate how, in a merozygote (partial diploid) with a complete (linear) chromosome from one parent and an incomplete chromosome from the other (three examples are shown, with the incomplete chromosome coming from one parent or the other and representing various regions of the chromosome), only even-numbered crossovers (double crossovers are shown here) yield viable recombinants with a complete chromosome (except in the rare cases where the incomplete chromosome includes an end). It follows that almost all recombinant progeny inherit both chromosome ends from the same parent; in other words the ends are 100% linked, thereby yielding map circularity.

the same cross. It turned out that the region of heterozygosity in a particular heteroclone could cover any continuous arc of the linkage map. On the assumption that this region represented an uninterrupted segment of the chromosome, the conclusion was that the chromosome lacked *constant* ends (Hopwood, 1966b). It was provisionally assumed to be circular pending direct evidence, but might have been circularly permuted instead. It was a very long time before we knew more than this!

The three phases of *Streptomyces* genetics

In vivo genetics was the order of the day for all organisms until the recombinant DNA revolution, starting in 1974, opened the way for the *in vitro* era, and changed genetics for ever. Methods for routine DNA sequencing, developed in 1977, led in turn to the *in silico* phase, based primarily on the explosion of databases and ever more sophisticated sequence comparisons. It took a little longer for these developments to take hold in *Streptomyces*. Although the phases overlapped of course, I shall regard the *in vivo* phase as occupying the rest of the 1960s and the 1970s; the *in vitro* phase as dominating the 1980s; and the 1990s seeing the rise of *in silico* *Streptomyces* genetics.

In skimming over developments in *Streptomyces* genetics since 1965, I have inevitably made subjective choices, not to say parochial ones [there is certainly a bias in favour of *S. coelicolor* A3(2)], even though I have tried to include in Tables 1–3 appropriate discoveries from other laboratories. I hope the choice will be viewed indulgently by those whose work I have omitted to mention.

The *in vivo* years

The major discoveries of the *in vivo* years are summarized in Table 1. An intriguing feature of the chromosome first observed in the mid-1960s, but still awaiting evaluation, is the indication of an ancestral

Table 1. *Streptomyces* genetics 1: the *in vivo* years

Year	Discovery	Reference
1955–57	Genetic recombination	Sermonti & Spada-Sermonti (1955), Alikhanian & Mindlin (1957), Braendle & Szybalski (1957), Hopwood (1957), Saito (1957)
1958	Rudimentary linkage analysis	Hopwood (1958, 1959)
1960	Heteroclones discovered	Sermonti <i>et al.</i> (1960), Hopwood <i>et al.</i> (1963)
1965	Single (circular) linkage group	Hopwood (1965b)
1967	Ancestral genome duplication? Discovery of A-factor (in <i>S. griseus</i>)	Hopwood (1967a) Khokhlov <i>et al.</i> (1967)
1969–73	The SCP1 plasmid and fertility	Hopwood <i>et al.</i> (1969), Vivian & Hopwood (1971, 1973), Vivian (1971), Hopwood & Wright (1973b)
1970	Discovery of ϕ C31 and <i>S. lividans</i> 66	Lomovskaya <i>et al.</i> (1970)
1970–72	Morphological mutants mapped	Hopwood <i>et al.</i> (1970), Chater (1972)
1974	Protoplast formation and regeneration	Okanishi <i>et al.</i> (1974)
1975	First CCC plasmid (SCP2) isolated Methylenomycin genes are on SCP1	Schrempf <i>et al.</i> (1975) Kirby <i>et al.</i> (1975), Kirby & Hopwood (1977)
1976	Antibiotic-pathway mutants mapped to the chromosome	Wright & Hopwood (1976b)
1977	Recombination via protoplast fusion	Hopwood <i>et al.</i> (1977), Baltz (1978)
1978	Protoplast transformation Plasmid-induced ‘pocks’	Bibb <i>et al.</i> (1978) Bibb <i>et al.</i> (1978)
1979	Transduction discovered (in <i>S. venezuelae</i>) Linear plasmids discovered (in <i>S. rochei</i>)	Stuttard (1979) Hayakawa <i>et al.</i> (1979)
1981	Integrating plasmids discovered	Bibb <i>et al.</i> (1981)

duplication of the genome. I noticed a tendency for genes for different steps in the same metabolic pathway to lie opposite one another on the circular linkage map. I proposed crossing-over between sister chromosomes in the same hyphal compartment to generate an ancestral double-sized circle, with all gene clusters duplicated on opposite sides of the map. Progressive loss of (most) duplicate genes would then leave some members of a cluster on each side (Fig. 7; Hopwood, 1967a). It will be fascinating to see if the current genome sequencing project (see below) reveals the kind of ‘mirror-image’ patches of residual sequence identity that might be expected on such a model.

1967 also saw the publication of a discovery that few people (including me) appraised correctly at the time – partly because it was made in the Soviet Union and reported in the Russian literature – but which is now seen as seminal. This was the discovery of A-factor as a diffusible molecule required, in minute concentrations, to activate streptomycin production and sporulation in *Streptomyces griseus* (Khokhlov *et al.*, 1967). A *tour de*

force of natural product chemistry by the Khokhlov group led to chemical characterization of this hormone-like molecule as a novel γ -butyrolactone (Fig. 8; Kleiner *et al.*, 1976). The later rediscovery of the Russian work, and its enormous development in *S. griseus*, *Streptomyces virginiae* and *S. coelicolor*, primarily by the groups of Teruhiko Beppu and Sueharu Horinouchi and of Yasuhiro Yamada (Hara & Beppu, 1982; Yamada *et al.*, 1987), has been one of the most significant facets of *Streptomyces* genetics over recent years (e.g. Onaka *et al.*, 1998).

A second highly significant development also took place in Moscow in the late 1960s. This was the penetrating work of Natalia Lomovskaya and her collaborators on the ϕ C31 temperate bacteriophage (Fig. 9; Lomovskaya *et al.*, 1970), which also launched its favoured host, *Streptomyces lividans* 66, on the world stage. Her generous sharing of her work and strains with Western scientists – including in particular Keith Chater at John Innes – at a difficult time for Russian geneticists has had far-reaching benefits.

Fig. 7. A possible explanation for map symmetry in *S. coelicolor* A3(2). An ancestral (circular) chromosome (i) has three clusters of biosynthetic genes – say A for arginine, H for histidine, T for tryptophan – each consisting of three genes, a, b, c. A crossover between sister chromosomes in the same mycelial compartment gives rise to a double-sized circle, on which duplicate clusters lie opposite to each other (ii). This dimer would be unstable: a further crossover would restore it to the monomeric state. However, the dimer could become stabilized by the accumulation of even a small number of recessive lethal mutations which would render the monomeric chromosomes inviable. Further mutations, including deletions, would then arise to leave, say, one copy of the A cluster with genes a and c, and the opposite copy with gene b (iii). Sometimes the whole of one cluster (for example T) might be lost, leaving an 'unpaired' cluster. See also Hopwood (1967a).

Fig. 8. The A-factor, first identified in *S. griseus*, by Khokhlov and his collaborators (Khokhlov *et al.*, 1967; Kleiner *et al.*, 1976).

Meanwhile, the varying fertility status of substrains of A3(2) was beginning to be interpreted. It became apparent that, just as in *E. coli*, plasmid sex factors were at work and that they had assumed different states in different strains: autonomous, integrated, primed and absent. I had noticed that when progeny selected from

Fig. 9. The ϕ C31 temperate actinophage (kindly provided by T. Clayton, The John Innes Centre, Norwich, UK).

my early crosses between mutants of the wild-type were crossed together they produced higher frequencies of recombinants than these early crosses themselves, but had not realized the significance of this difference. Sermonti had done so, and had begun to study the phenomenon (Sermonti & Casciano, 1963). In Glasgow, we obtained a particularly surprising result in crosses between so-called NF and UF strains (later interpreted as having an integrated SCP1 plasmid and no SCP1 respectively: Vivian & Hopwood, 1970; Vivian, 1971). These crosses produced recombinants so abundantly that they could be analysed non-selectively, a rare occurrence in a bacterium (Hopwood *et al.*, 1969).

The plasmid nature of SCP1 was deduced genetically. In spite of some raised eyebrows, because we had not identified the element physically, this did not render the conclusion invalid in view of the original definition of the word 'plasmid', which did not refer to its physical nature (Lederberg, 1952). However, we needed to isolate SCP1, and it long resisted isolation, notwithstanding valiant attempts by Janet Westpheling and others (see Hopwood *et al.*, 1979). The reason, discovered nearly ten years later by Haruyasu Kinashi (Kinashi *et al.*, 1987), who exploited the newly invented pulsed-field gel technology, is that SCP1 is both *large* (350 kb) and *linear*. With hindsight, we should have taken more seriously the possibility that SCP1 was linear, because linear plasmids small enough to be revealed by conventional technology had been found as early as 1979 (Hayakawa *et al.*, 1979). This was another momentous development, the full significance of which became apparent only much later when the widespread occurrence in *Streptomyces* of linear replicons, including the chromosome, was realized.

Alan Vivian (1971) had found that SCP1⁺ strains inhibited SCP1⁻ strains through the agency of a diffusible molecule, but only later was this found to be a small molecule (i.e. not a protein like a bacteriocin) with

Fig. 10. Assay of methylenomycin production. Agar plugs of five SCP1⁺ and two SCP1⁻ *S. coelicolor* A3(2) strains were placed on a Petri plate spread with a lawn of *Bacillus mycoides*. After incubation, the SCP1⁺ plugs are surrounded by zones of inhibition of the lawn.

a wide spectrum of activity (Fig. 10) and so it could be called an antibiotic (Kirby *et al.*, 1975), later identified as methylenomycin (Wright & Hopwood, 1976a). This, the first example of genetic localization of antibiotic-biosynthetic genes, led to much speculation that plasmid determination of antibiotic production might be widespread, if not typical (e.g. Umezawa, 1977). Ironically, methylenomycin remains, after 20 years, the only unambiguous example of a *Streptomyces* antibiotic specified by a plasmid. The very next example – actinorhodin in *S. coelicolor* (Wright & Hopwood, 1976b) – implicated chromosomal genes, later shown to be clustered (Rudd & Hopwood, 1979), and this instead has turned out to be the paradigm.

In parallel with the mapping of antibiotic-biosynthetic genes, developmental mutants, defining the other special feature of *Streptomyces* biology, were also mapped in the early 1970s. One of them – S48, later named *bldA* (see below) – had actually been used as a textbook example of genetic mapping even earlier (Hopwood, 1967b), but systematic classification and mapping of *whi* (white colony) mutants came a little later (Hopwood *et al.*, 1970; Chater, 1972), and the *bld* mutants were given similar treatment in 1976 (Merrick, 1976).

Meanwhile, a second plasmid had surfaced in *S. coelicolor*; this proved to be a conventional, moderate-sized, CCC molecule, amenable to isolation by a fairly standard protocol (Schrempf *et al.*, 1975). SCP2 was largely responsible for the fertility in crosses that could not be attributed to SCP1 (Bibb *et al.*, 1977); a variant form of the plasmid, called SCP2*, was an even better sex factor. The discovery of SCP2* was an essential stepping stone towards introducing plasmid DNA into *Streptomyces* hosts by transformation, thereby opening the way for gene cloning in these organisms. The transformation procedure devised (Bibb *et al.*, 1978)

Fig. 11. Pocks produced by the growth of a few plasmid-containing spores of a strain of *S. lividans* in a background of a plasmid-free *S. lividans* culture. Left: pocks produced by a pIJ101 derivative with wild-type 'spread' genes; each pock is a few millimetres in diameter and represents the area of the indicator culture into which the plasmid has migrated. Right: pocks produced by a plasmid mutated in the 'spread' genes for putative intramycelial migration; the much reduced size of the pocks was the first clue to the existence of such genes. See also Kieser *et al.* (1982).

depended on the earlier development of protoplast fusion, which gave rise to enormously high frequencies of genetic recombination without the need for a plasmid vector (Hopwood *et al.*, 1977; Baltz, 1978). These studies, in turn, could not have been made without the pioneering work of Masanori Okanishi and his colleagues, who painstakingly investigated the factors necessary for good protoplast formation and, even more crucially, their regeneration (Okanishi *et al.*, 1974).

Plasmid biology was also beginning to develop as the 1970s were coming to an end. The marker used by Bibb *et al.* (1978) to track transformants by SCP2* was 'pock' formation: transformants receiving SCP2* gave rise to small, circular areas of retarded growth in a lawn of the plasmid-free strain. Originally this effect was assumed to be an actual killing of the strain by the mating process, and was likened to lethal zygosis in *E. coli* (Skurray & Reeves, 1973). An even earlier notion, when pocks were seen in matings between *S. coelicolor* carrying SCP1 and *S. lividans* (Hopwood & Wright, 1973a), was that inhibition of the *S. lividans* lawn was due to the diffusible agent that was subsequently identified as methylenomycin. Only later, when plasmid mutants altered in pock formation were isolated (Fig. 11: Kieser *et al.*, 1982), was the phenomenon interpreted (we hope correctly, but direct proof is still lacking) as a manifestation of the migration of a conjugative plasmid *within the mycelium* of the recipient. This process was described as plasmid 'spreading' and became capable of molecular analysis only in the *in vitro* years.

Pocks meanwhile led to the discovery of a further major class of *Streptomyces* plasmids. The first example, SLP1, was revealed when pocks were found in a lawn of *S.*

Table 2. *Streptomyces* genetics 2: the *in vitro* years

Year	Discovery	Reference
1980	First genes cloned in <i>Streptomyces</i>	Bibb <i>et al.</i> (1980), Suarez & Chater (1980), Thompson <i>et al.</i> (1980)
1981	First report of amplified DNA segments	Robinson <i>et al.</i> (1981)
1982	Isolation of promoter sequences	Bibb & Cohen (1982)
1983	First <i>Streptomyces</i> gene sequenced (<i>aph</i> of <i>S. fradiae</i>)	Thompson & Gray (1983)
	Antibiotic-pathway genes cloned	Chater & Bruton (1983), Feitelson & Hopwood (1983), Gil & Hopwood (1983)
1984	Complete antibiotic pathway (<i>act</i>) cloned	Malpartida & Hopwood (1984)
	FRAME program for gene recognition	Bibb <i>et al.</i> (1984)
1985	Developmental gene (<i>bldA</i>) cloned	Piret & Chater (1985)
	'Hybrid' antibiotics	Hopwood <i>et al.</i> (1985a)
	Sigma factor heterogeneity	Westpheling <i>et al.</i> (1985), Buttner <i>et al.</i> (1988)
	Multiple promoters for the same gene	Bibb <i>et al.</i> (1985), Buttner <i>et al.</i> (1987)
	Linkage of antibiotic-biosynthetic, -regulatory and -resistance genes	Chater & Bruton (1985)
	John Innes Laboratory Manual published	Hopwood <i>et al.</i> (1985b)
1987	First <i>Streptomyces</i> transposon discovered	Chung (1987)
1987/88	Operon structure (<i>gal</i> and <i>gyl</i>)	Fornwald <i>et al.</i> (1987), Smith & Chater (1988)
1989	Translation without Shine–Dalgarno sequences	Janssen <i>et al.</i> (1989)
	Conjugation between <i>E. coli</i> and <i>Streptomyces</i>	Mazodier <i>et al.</i> (1989)

lividans after contact with an *S. coelicolor* strain devoid of SCP1 and SCP2 (Bibb *et al.*, 1981). The pocks yielded a family of SLP1 plasmids of varying size. SLP1 was evidently integrated in the *S. coelicolor* chromosome and could loop out to form a conjugative plasmid. A large family of such elements was soon discovered, of which the most extensively studied, along with SLP1 (e.g. Shiffman & Cohen, 1993), is pSAM2 from *Streptomyces ambofaciens* (e.g. Sezonov *et al.*, 1995).

As the 1970s came to an end, generalized transduction was added to the other two modes of gene transfer – by plasmid-mediated conjugation and by transformation (albeit artificial) – when Colin Stuttard described a phage, SV1, that could transduce markers in *Streptomyces venezuelae* (Stuttard, 1979). Unfortunately, SV1 did not work in any other species; and the second example of general transduction, by SF1 in *Streptomyces fradiae* (Chung, 1982), was also taxonomically circumscribed. Only very recently has a system of general transduction for *S. coelicolor* and *S. lividans* been developed (J. Westpheling, personal communication), thereby promising to fill a significant gap in the genetic tool-kit.

The *in vitro* years

The major achievements of the *in vitro* years are summarized in Table 2. The 1980s opened momentarily for *Streptomyces* genetics with the first reports of gene cloning in *S. coelicolor* and *S. lividans* (Bibb *et al.*, 1980; Suarez & Chater, 1980; Thompson *et al.*, 1980). Of the genes cloned in these first experiments, several conferring antibiotic resistances soon established themselves as versatile tools in vector development; the *tsr* gene, for thiostrepton resistance (Thompson *et al.*, 1980), is perhaps the most famous, having been used as a selective marker on a multitude of *Streptomyces* cloning vectors (a mixed blessing perhaps, because this antibiotic is not the easiest to come by!).

The developments in *Streptomyces in vitro* genetics that followed during the 1980s may be divided into two broad groups. One includes the beginnings of an understanding of basic features of the molecular biology and physiology of the organisms, such as gene and operon structure, and the control of transcription, translation and primary metabolism; the other represents the use of gene cloning to understand the molecular genetics of some of the special features of *Streptomyces*

biology, especially antibiotic production and the developmental cycle.

In the first category, developments were coming thick and fast by the mid-1980s. *Streptomyces* promoter sequences were isolated (Bibb & Cohen, 1982) and soon after that, the first *Streptomyces* gene was sequenced (Thompson & Gray, 1983). Not surprisingly, this gene sequence revealed an extraordinarily high proportion of codons ending in G or C, and this bias, typical of *Streptomyces* genes, was exploited in the FRAME program (Bibb *et al.*, 1984), which remains an extremely useful tool for finding genes (and frameshift errors!) in DNA sequences. Soon, promoters were sequenced and found to be both heterogeneous and abundant: many genes turned out to have more than one transcription-start site (e.g. Bibb *et al.*, 1985; Buttner *et al.*, 1987), a finding that is still not understood in any detail but which may reflect the need for subtly modulated expression at different stages in the life cycle or in different physiological states.

Pioneering studies revealed multiple forms of RNA polymerase holoenzyme in *S. coelicolor* (Westpheling *et al.*, 1985; Buttner *et al.*, 1988), establishing this as a potential strategy for differential regulation of different subsets of genes in *Streptomyces*, following the *Bacillus subtilis* paradigm (Losick & Pero, 1981). The number of different sigma factors in *S. coelicolor* has increased steadily over the years, including the remarkable and still only partially understood presence of four sigma factors that strongly resemble the vegetative *E. coli* and *B. subtilis* form (Tanaka *et al.*, 1988); only one of these is essential (Buttner, 1989). Currently, the number of known or inferred alternative sigma factors in *S. coelicolor* runs to at least 20 (M. J. Buttner, G. Kelemen & M. Paget, personal communication): even more than in *B. subtilis*.

Another surprising discovery was the relative abundance of *Streptomyces* genes that must be translated without a conventional Shine–Dalgarno sequence, because there is no untranslated leader on the mRNA before the start codon (Janssen *et al.*, 1989). This provided one of the rather rare examples of a basic aspect of molecular biology that was brought to prominence in *Streptomyces* rather than in *E. coli*.

The first examples of *Streptomyces* operons to be rigorously defined reflect aspects of primary metabolism, and specify the proteins needed for the utilization of galactose and glycerol (Fornwald *et al.*, 1987; Smith & Chater, 1988). Both are subject to specific induction as well as generalized carbon-catabolite repression by glucose (Mattern *et al.*, 1993; Hindle & Smith, 1994). This global regulatory control has itself turned out to differ fundamentally in mechanism from the *E. coli* paradigm, with an involvement of glucose kinase, but not of cyclic AMP (Hodgson, 1982; Angell *et al.*, 1994).

In the second category, the 1980s saw the beginning of our understanding of the molecular genetics of antibiotic production and its manipulation. The first antibiotic-biosynthetic genes were cloned, by a variety of methods

Fig. 12. Zones of inhibition by methylenomycin of a lawn of an SCP1⁺ strain of *S. coelicolor* A3(2) by blocks cut from NF cultures (carrying SCP1 in the chromosome) lysogenized by insert-directed recombination with ϕ C31 phage vectors carrying various segments of the *mmy* gene cluster. The left-hand culture shows the wild-type level of methylenomycin production, whereas the right-hand culture shows increased production caused by 'insertional mutagenesis' of what was thus identified as a negatively acting regulatory gene. The middle culture shows partial de-repression caused by inactivation of another gene. Combined with the identification of a resistance gene and biosynthetic genes in the cluster, this finding established the paradigm that antibiotic-biosynthetic, -resistance and -regulatory genes are clustered. See also Chater & Bruton (1985).

(Chater & Bruton, 1983; Feitelson & Hopwood, 1983; Gil & Hopwood, 1983), and soon genes for a whole pathway, the *act* genes for actinorhodin biosynthesis, were isolated and expressed in a different *Streptomyces* host (Malpartida & Hopwood, 1984). Just as loss of the blue colour of actinorhodin had been instrumental in the isolation and *in vivo* mapping of antibiotic-pathway genes (Rudd & Hopwood, 1979), so its reacquisition was crucial to the isolation and *in vitro* mapping of the genes (Malpartida & Hopwood, 1984, 1986). Soon, colour was exploited again, this time to produce a hybrid antibiotic by genetic engineering; when specific segments of the *act* gene cluster were introduced on a plasmid vector into the producer of the brown antibiotic medermycin, a beautiful purple culture arose and was found to be making mederrhodin, a compound with structural features of both medermycin and actinorhodin (Hopwood *et al.*, 1985a).

While cloning the *act* genes demonstrated close linkage of biosynthetic structural genes, the finding that *Streptomyces parvulus* containing the *act* cluster produced actinorhodin without killing itself (Malpartida & Hopwood, 1984) provided indirect evidence that a resistance gene had also been cloned; the basis of actinorhodin self-resistance, while probably in some way reflecting antibiotic export (Bystrykh *et al.*, 1996), is still not well understood. In contrast, the methylenomycin gene cluster, on SCP1, was proved to contain resistance as well as biosynthetic and regulatory genes. This followed from the recognition of a specific methylenomycin-resistance gene – the very first *Streptomyces* gene to be cloned in *Streptomyces* (Bibb *et al.*, 1980) – as part of the DNA that caused methylenomycin production in a non-producing host, and overproduction when a deduced regulatory region was inactivated (Fig. 12; Chater & Bruton, 1985).

Table 3. *Streptomyces* genetics 3: the *in silico* years

Year	Discovery	Reference
1987	<i>bldA</i> is a tRNA gene	Lawlor <i>et al.</i> (1987)
1988	Complete sequence of pIJ101	Kendall & Cohen (1988)
1989	<i>whiG</i> encodes a developmental sigma factor	Chater <i>et al.</i> (1989)
	Type II polyketide synthase organization	Bibb <i>et al.</i> (1989), Sherman <i>et al.</i> (1989)
1990–91	Modular polyketide synthases (<i>ery</i> in <i>S. erythraea</i>)	Cortes <i>et al.</i> (1990), Donadio <i>et al.</i> (1991)
1992	Physical map of the <i>S. coelicolor</i> chromosome	Kieser <i>et al.</i> (1992)
1993	The chromosome is linear	Lin <i>et al.</i> (1993)
	Combinatorial biosynthesis of polyketides	McDaniel <i>et al.</i> (1993)
1994	Model for linear plasmid (and chromosome) replication	Chang & Cohen (1994)
	ECF subfamily of sigma factors discovered	Lonetto <i>et al.</i> (1994)
	Serine/threonine protein kinase (AfsK)	Matsumoto <i>et al.</i> (1994)
1996	Ordered cosmid library for <i>S. coelicolor</i> chromosome	Redenbach <i>et al.</i> (1996)
1997	<i>S. coelicolor</i> genome sequencing project starts	www.sanger.ac.uk/Projects/S_coelicolor
	SARPs recognized as OmpR relatives	Wietzorrek & Bibb (1997)
1999	Complete sequence of ϕ C31 phage	Smith <i>et al.</i> (1999)
	2Mb (out of 8Mb) of <i>S. coelicolor</i> genome sequenced (January)	www.sanger.ac.uk/Projects/S_coelicolor

On the chromosome front, the 1980s saw the flowering of a whole new subfield of *Streptomyces* genetics stemming from the report by Robinson *et al.* (1981) of the remarkable amplification of specific DNA segments. This phenomenon, which was later found to be associated with large (up to 1000 kb) deletions, has been studied in many strains by several groups (e.g. Schrepff, 1982; Ono *et al.*, 1982; Altenbucher & Cullum, 1984). The apparent association of deletion and amplification cycles with the end regions of the linear chromosome, although probably not in an obligate way, has recently given the topic a new impetus (e.g. Lin & Chen, 1997; Volff & Altenbuchner, 1998).

Two further techniques were added to the toolbox for *Streptomyces* genetic manipulation at the end of the 1980s. The first *Streptomyces* transposon was identified in 1987. This was a Tn3-type element found in *S. fradiae* when it jumped on to the plasmid prophage of SF1, the transducing phage already referred to (Chung, 1987). A second was engineered from a naturally occurring insertion sequence in *S. lividans* (Solenberg & Burgett, 1989). The use of transposons in *Streptomyces* has lagged behind that in many other bacteria. However, the most promising seem now to be those isolated from other bacteria, such as *Mycobacterium* (Smith & Dyson, 1995), or even Tn5 (Volff & Altenbuchner, 1997). The second major advance was the demonstration of conju-

gal transfer of plasmids from *E. coli* to *Streptomyces* (Mazodier *et al.*, 1989). The trick was to provide *E. coli* with a bifunctional plasmid containing a copy of *oriT*, as well as with mobilizing genes on the chromosome or on a second plasmid. This system has become a very useful one for the straightforward transfer of genes into *Streptomyces* (e.g. Bierman *et al.*, 1992).

As a manifestation of all the activity in *Streptomyces in vitro* genetics in the 1980s, as well as the continued use of *in vivo* techniques, the John Innes Manual was published in 1985 (Hopwood *et al.*, 1985b). Judging by the number of research papers citing its use over the next decade, it filled a major gap. Now it is badly out of date, but we hope that the second edition, in preparation since 1994, will appear before the next millennium dawns (T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater & D. A. Hopwood, unpublished).

The *in silico* years

The main developments of the *in silico* years are summarized in Table 3. If we define *in silico* genetics as the drawing of conclusions about the functions of genes from comparisons between a newly determined DNA sequence and sequences already in the databases, this phase of *Streptomyces* genetics probably began with the sequencing of the *S. coelicolor bldA* gene in 1987.

Fig. 13. Organization of gene clusters for type II polyketide synthases (PKS) that direct biosynthesis of the carbon skeletons of aromatic polyketides in streptomycetes and other actinomycetes. Early examples (*act* and *tcm* for actinorhodin and tetracenomycin) were cloned by complementation of blocked mutants, but most of the others were isolated by using the *act* PKS genes as hybridization probes. The genes have been used extensively for the generation of 'unnatural natural products' by 'combinatorial biosynthesis' (e.g. McDaniel *et al.*, 1995). Reprinted with permission from Hopwood (1997). Copyright (1997) American Chemical Society.

Mutations in *bldA* abolish aerial-mycelium formation (hence the name *bald*) and also prevent production of the four known *S. coelicolor* antibiotics under certain nutritional conditions; this phenotype was so pleiotropic that the gene was thought likely to act at a central point in decision-making during the life cycle. Sure enough, the sequence showed that *bldA* could not encode a protein but rather would produce a typical tRNA with the anti-codon UUA ($\equiv$ TTA in DNA) (Lawlor *et al.*, 1987). So was born the concept of a developmental switch that is dependent on the absence of the rare TTA codon for leucine from all genes required for vegetative growth, but present in a limited number of genes expressed post-exponentially (Leskiw *et al.*, 1991). This idea was strikingly illustrated by the finding of a TTA codon in the 5' region of *actII*-ORF4, the gene for the pathway-specific activator of the actinorhodin cluster: mutation of this codon to TGA restored antibiotic production in a *bldA* background (Fernández-Moreno *et al.*, 1991). The genome-sequencing project for *S. coelicolor* is providing a potentially complete inventory of genes containing TTA codons, and therefore making up a regulon controlled

by *bldA*: the current prediction is about 100 genes (K. F. Chater, personal communication).

A second striking example of the power of *in silico* comparison came soon after: the sequence of the *whiG* gene, mutation of which causes the aerial mycelium to continue indeterminate growth and fail to switch to metamorphosis into chains of spores, showed it to encode a sigma factor (Chater *et al.*, 1989), thereby establishing RNA polymerase heterogeneity as a strategy in *Streptomyces* development, recollecting the *B. subtilis* story (Losick & Pero, 1981). Again, sequencing and *in silico* analysis had short-circuited perhaps years of experimental attempts to elucidate the role of a developmental gene. This approach continues to bear fruit (e.g. Chater, 1998).

In the field of antibiotic biosynthesis, *in silico* genetics soon began to play an equally decisive role. Sequencing of the DNA regions responsible for the early carbon-chain-building steps towards the aromatic polyketides granaticin and tetracenomycin revealed that the enzymes involved – the first bacterial polyketide synthases to be studied by molecular genetics – were organized like

Fig. 14. The modular erythromycin polyketide synthase and the 'assembly-line' model of biosynthesis leading to 6-deoxyerythronolide B, and on to erythromycin A through the action of tailoring enzymes. Reprinted with permission from Hopwood (1997). Copyright (1997) American Chemical Society.

those of a typical bacterial fatty acid synthase, strikingly confirming the idea that polyketide and fatty acid synthesis are homologous processes. The synthases were clearly so-called type II enzymes, consisting of separate subunits (Sherman *et al.*, 1989; Bibb *et al.*, 1989); this turned out to be the rule for aromatic polyketides (Fig. 13). In contrast, similar studies on the molecular genetics of erythromycin biosynthesis in *Saccharopolyspora erythraea* revealed a type I polyketide synthase organization, in which large proteins carry the different enzymic functions as a linear array of domains, an organization hitherto known only in eukaryotes. Even more remarkably, the sequencing revealed multiple sets, or modules, of domains (Fig. 14) that would together form an 'assembly line' for biosynthesis of the polyketide backbone (Cortes *et al.*, 1990; Donadio *et al.*, 1991). I have likened this discovery to that of the deduction of the mode of DNA replication directly from the Watson-Crick structure (Hopwood, 1997); the 'assembly line' model for complex polyketide biosynthesis had to be experimentally verified, but it arose directly from the determination of the gene sequences. These discoveries about the aromatic and complex polyketides in turn opened the way for the burgeoning field of 'combinatorial biosynthesis' of 'unnatural natural products' by the genetic engineering of polyketide synthases (McDaniel *et al.*, 1993, 1999; Khosla & Zawada, 1996; Hopwood, 1997).

Some other interesting examples of the power of *in silico* analysis in *Streptomyces* genetics are as follows: the first bacterial serine/threonine protein kinase (hitherto a

eukaryotic preserve) was deduced from the sequence of *afsK* of *S. coelicolor* and *S. lividans* (Matsumoto *et al.*, 1994); a new subfamily of sigma factors (Fig. 15), the ECF subfamily (for Extra Cytoplasmic Function), was discovered when the newly determined sequence of the *S. coelicolor sigE* gene was compared with other sequences in the databases (Lonetto *et al.*, 1994); and the discovery of homology between the *actII-ORF4* family of pathway-specific regulators (SARP, the Streptomyces Antibiotic Regulatory Proteins) and OmpR-type DNA-binding proteins uncovered a characteristic series of DNA motifs recognized by such proteins (Wietzorrek & Bibb, 1997).

The above examples of *in silico* *Streptomyces* genetics dealt with the sequencing of an individual gene or a group of clustered genes that had previously been identified by *in vivo* or *in vitro* genetics, rather than with the sequencing of an entire replicon followed by systematic *in silico* analysis of the complete sequence: in other words with 'genomics'. The genomics phase of *Streptomyces* genetics began with the sequencing of some plasmids. Although a plasmid of 3706 bp, pSB24.1, had been sequenced as early as 1985 (Bolotin *et al.*, 1985), the first sequence to make an impact was that of pIJ101 (Kendall & Cohen, 1988), because considerable *in vivo* and *in vitro* genetics had been done with this plasmid since its discovery in 1982 (Kieser *et al.*, 1982). The 8830 bp sequence of pIJ101 revealed seven putative genes and immediately began to clarify the genetic control of plasmid transfer, both intermycelial and, putatively, intramycelial by the so-called *spd* ('spread')

Fig. 15. Phylogenetic relationships between the ECF subfamily and other RNA polymerase sigma factors. The new subfamily, with representatives in nearly all eubacteria, was recognized after the discovery of SigE from *S. coelicolor* A3(2) and greatly extended the span of the whole family of proteins. Reprinted with permission from Lonetto *et al.* (1994). Copyright (1994) National Academy of Sciences, USA.

genes, as well as regulatory circuits for the control of replication and transfer (Fig. 16). The 41489 bp sequence of the model *Streptomyces* phage, ϕ C31, has been completed only very recently (Smith *et al.*, 1999), and has led to many interesting conclusions. For example, all of the genes are orientated in the same direction except for one which, together with its promoter and operator, appears to have been inserted from elsewhere. There is much evidence for horizontal gene transfer involving other genes too, including perhaps genes shared by ϕ C31 and some eukaryotic viruses. Another fascinating find in ϕ C31 is a homologue of a plasmid 'spread' gene, suggesting a relationship between the putative intramycesial migration of plasmids and the movement of phage genomes between mycesial compartments during the lytic phase.

The *Streptomyces* chromosome, at 8 Mb, is enormous compared with most plasmids and phages so chromosomal genomics has taken longer to come on stream. A crucial enabling step was the use of PFGE to construct a restriction map of the *S. coelicolor* A3(2) chromosome for the two rare-cutting enzymes *Ase*I and *Dra*I, and to relate the physical to the genetic map (Kieser *et al.*, 1992). The result was gratifying in that no examples

Fig. 16. A map of *Streptomyces* plasmid pIJ101, based on the complete nucleotide sequence (Kendall & Cohen, 1988), with other information added. Note in particular the single gene (*tra*) for intermycesial transfer and the spread genes (*spdA*, *B*) potentially involved in intramycesial migration. See also Hopwood & Kieser (1993).

were found in which gene order deduced from the results of *in vivo* crosses was contradicted by the physical evidence: even the relative spacing of the genes was quite well matched on the genetic and physical maps, except for an *underestimation* of the genetic length of the 3 o'clock 'silent region'. This region was thus confirmed as a long stretch of DNA almost devoid of recognized genes, rather than a region of modest length in which the genetic distance was inflated by unusually frequent crossing-over. Physical linkage across both 'silent regions' (the shorter 9 o'clock one having in any case been partially filled by markers) appeared to have been confirmed, thereby solving the age-old question of chromosome circularity: the chromosome was concluded to be circular.

Hardly had the Kieser *et al.* (1992) paper been published than things took a dramatic turn. Carton Chen came on sabbatical to the John Innes Centre from the National Yang-Ming University in Taipei in the summer of 1992, with the first evidence for a linear chromosome in *S. lividans*, and he and Helen Kieser, with constant input from Yi-Shing Lin back in Taipei, clearly established this (Lin *et al.*, 1993). Pretty soon, linearity was shown also for the *S. coelicolor* chromosome. How had this been missed? It turned out that a mistake had been made in positioning one of the *Dra*I sites and so a point where apparent *Dra*I and *Ase*I sites coincided had been overlooked (this was the position of the chromosome ends); and long terminal repeats, later estimated to be >60 kb, at either end of the chromosome had led to

Fig. 17. Combined genetic and physical map of the *S. coelicolor* chromosome. The positions of markers on the outside of the circle come from their locations on the cosmid contig. Markers inside the circle have been mapped only genetically; their approximate positions relative to the physically mapped markers were determined by interpolation. Sizes of the *Asel* and *DraI* fragments are in kb. ● chromosomal 'telomeres'; ◆, *oriC*. Reprinted with permission from Redenbach et al. (1996). Copyright (1996) Blackwell Sciences Ltd.

hybridization of a probe taken from a point near to one end, to *both* ends, thereby mimicking a physical continuity across the 3 o'clock region. Carton Chen has vividly described the whole story, and especially the excitement of the hunt for the chromosome ends (Chen, 1997). The discovery has helped to change our ideas about bacterial chromosomes. It was also linked to elegant experiments on linear plasmids which proposed a model for *Streptomyces* chromosome replication in which conventional bidirectional replication takes place from a typical, centrally located *oriC* (Calcutt & Schmidt, 1992; Zakrzewska-Czerwinska & Schrepf, 1992), followed by 'patching' replication primed by proteins attached covalently to the free 5' ends of the chromosome to fill the gap left by removal of the RNA primer for the last Okazaki fragment on each discontinuous strand (Chang & Cohen, 1994).

The next step towards genomics was the preparation by Matthias Redenbach of a Supercos library of *S. coelicolor* chromosomal DNA and the selection from it, in a fruitful collaboration involving Kaiserslautern, Hiroshima and Norwich, of a minimal set of some 320 cosmids that form an overlapping library of clones covering the complete chromosome, except for three short gaps (Redenbach et al., 1996). More than 170 genes, gene clusters and other genetic elements were located on the set of cosmids, mainly by hybridization to specific cosmids or the overlaps between pairs of adjacent cosmids, to form a detailed genetic and physical map (Fig. 17). This immediately became a valuable resource to help in gene mapping and gene isolation for the *Streptomyces* genetics community. Even more importantly, it became the jumping-off point for the genome-sequencing project which was initiated at the

Sanger Centre at Hinxton, Cambridge in August 1997 with funding from the UK Biotechnology and Biological Sciences Research Council (BBSRC) (www.sanger.ac.uk/Projects/S_coelicolor). Collaborative funding from the Beowulf Genomics initiative of the Wellcome Trust in October 1998 has ensured that the complete sequence should be available by late 2000.

Already the sequence is proving enormously valuable as a source of information and genes. As at 15 January 1999, nearly 1.2 Mb of sequence is annotated, and has revealed 1045 protein coding sequences (CDSs). The mean density is therefore 1.14 kb per CDS, a very typical bacterial value. With a genome size of 8 Mb we predict a total of about 7000 genes, assuming a uniform density around the genome; in fact there is no reason to believe that the 'silent regions' near the ends of the chromosome are more sparsely populated by CDSs than the 'core' region; it is simply that a much higher proportion of genes in these regions is dispensable under laboratory conditions and/or are represented by 'redundant' sets of similar genes. The total number of genes is some 1.7 times that in bacteria like *E. coli*, *B. subtilis* and *Mycobacterium tuberculosis*, and rather more even than the eukaryote *Saccharomyces cerevisiae* with about 6000 genes. This raises the question: Why does *Streptomyces* have so many genes? Streptomycetes make antibiotics, and have a complex developmental cycle, so extra genes are needed, but how many?

Clusters of antibiotic-biosynthetic genes in actinomycetes range in size from about 20 kb or less for a relatively simple aromatic polyketide like actinorhodin to at least 90 or 100 kb for complex polyketides like rifamycin or rapamycin, where encoding the modular polyketide synthases alone requires 50–80 kb of DNA (Schwecke *et al.*, 1995; August *et al.*, 1998). Even though a given strain can make several different antibiotics, it is thus likely that genes encoding antibiotic-biosynthetic enzymes, and the associated genes for self-protection and pathway-specific activators, run to a few hundreds, not thousands. However, many other genes are probably involved more indirectly in modulating antibiotic production (Bibb, 1996).

The number of genes *directly* involved in determining the life cycle, including those currently recognized by the *bld* and *whi* genes, is also not likely to exceed a hundred or two (Chater, 1998), even though the range of genes directly involved in the transition to aerial mycelium production has been crucially expanded from the original set of *bld* loci (Merrick, 1976) by the isolation of others that seem to act via an extracellular signalling pathway involving a morphogenetic protein, SapB (Willey *et al.*, 1993). Again, the discovery of two phases of glycogen deposition and hydrolysis, at different stages of the life cycle, and controlled by two sets of paralogous genes (Bruton *et al.*, 1995), served to identify genes with a presumptive but indirect role in the developmental cycle which would not have been discovered by a simple search for mutants with a clear defect in morphogenesis.

In agreement with these ideas, the CDSs so far annotated

in the *S. coelicolor* genome sequence include a high proportion that would encode putative transcriptional regulators, sensors, transporters and clusters of genes likely to be dedicated to the uptake and utilization of nutrients (J. Parkhill, personal communication; www.sanger.ac.uk/Projects/S_coelicolor). Such genes could help the organism to respond appropriately to a soil environment that is highly variable in terms of physical, chemical, nutritional and biotic (competition) stresses. It will be a major challenge, when *in silico* genomics of *S. coelicolor* gives way to global functional analysis, to find out what all these genes are doing, not to mention the ~27% of CDSs with no significant database match. I look forward eagerly to this analysis of the *S. coelicolor* transcriptome, proteome and metabolome as the next phase of *Streptomyces* genetics.

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