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Sweet home actinomycetes: The 1999 MDS Panlabs Lecture

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For the past 25 years, I have devoted most of my research efforts to the application of molecular genetics to yield improvement and production of novel secondary metabolites in actinomycetes. My group at Lilly Research Laboratories worked with a variety of *Streptomyces* species and with strains of *Amycolatopsis* and *Saccharopolyspora*. We developed molecular genetic tools to manipulate actinomycete genes, and applied them to important secondary metabolites, including tylosin, daptomycin, vancomycin, chloroeremomycin, and spinosyns. In the early years, I helped establish recombinant DNA technology to manufacture mammalian proteins, and more recently, helped implement microbial genomics as a research tool for antibiotic discovery. In this paper, I review some highlights, primarily from the actinomycete work. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 79–88.

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Introduction

'*Two roads diverged in a yellow wood . . .*' Robert Frost

Twenty-five years ago I was working on the mechanisms of mutation in bacteriophage T4. As a graduate student in Jan Drake's laboratory at the University of Illinois, I developed transfection and transformation assays to study mutagenic mechanisms in bacteriophage T4 [2,3,14]. As a postdoctoral student in the same lab, I studied the mechanisms of heat- and acid-induced 'spontaneous' mutations, showing that cytosine deamination was mutagenic, and that depurination was the probable mechanism for a second mutational pathway [21,33]. I was very interested in this work, and had many more experiments planned when I received a call from Larry Day at Eli Lilly and Company. He wanted to know if I was interested in interviewing for a Senior Scientist position. The responsibility would be to develop genetic systems in *Streptomyces* to improve antibiotic production. My only experience with streptomycetes was the infrequent encounters with slow-growing fuzzy contaminants on Drake agar plates. So I asked Larry, 'What is known about the genetics of *Streptomyces*? Can you transform them? Do they have phages? Do they conjugate? How do they mutate?' Larry's answer was, 'Well, there's this guy David Hopwood in England. He has studied the genetics of *Streptomyces coelicolor*, and has demonstrated plasmid primes. That's about it.' Larry sent me the article on plasmid primes and I read it. I had several lengthy discussions with Larry over the phone, and finally convinced myself that I should interview for the job. These microbes had stimulated my curiosity. I met Claude Nash and Steve Queener, among others, during the interview, and was impressed by the work that they were pursuing. I accepted the job offer and embarked on the 'less traveled' actinomycete road.

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This year marks the 50th anniversary of the founding of SIM, and the 20th anniversary of my first SIM meeting. The late Paul Lemke invited me to organize a session on the genetics of streptomycetes for the 1979 meeting at Carnegie Mellon University in Pittsburg. These were the early days of streptomycete molecular genetics and of recombinant DNA technology. My session had talks on protoplast fusion (R Baltz), protoplast transformation (M Bibb), actinophage DNA (K Chater), and genetic instability (H Schrempf). Interestingly, two of the talks were chosen this year as Landmark Papers in Industrial Microbiology [12,31]. In another session, Ron Cape gave a talk entitled 'Molecular biology is finally being exploited—let me count some ways'. This was a time of uncertainty about the future impact of the new technologies, but the mood was optimistic. Twenty years later, it is clearer what the impacts are, and can yet be, but much work is still needed to fully exploit the molecular genetics of actinomycetes [13]. In this paper I summarize some of the work carried out in my laboratory during my years at Lilly and in collaboration with scientists at Dow AgroSciences. At the end, I will explain the title.

The early years (1974–1981)

When I joined the Antibiotic Development Division of Eli Lilly and Company in September of 1974, the company was working on fermentations of apramycin, capreomycin, cephalosporin C, erythromycin, monensin, naracin, hygromycin B, penicillin, tobramycin, tylosin and vancomycin. All but two of these secondary metabolites were produced by actinomycetes. Steve Queener helped convince me that tylosin production in *Streptomyces fradiae* would be a relevant model system to develop genetic and molecular genetic techniques because of tylosin's interesting chemistry and biosynthesis (Figure 1). Four related molecules were produced in tylosin fermentations: tylosin, relomycin, macrocin, and desmycosin; the latter two were potential precursors of tylosin. So I initiated mutation and recombination studies in *S. fradiae*.

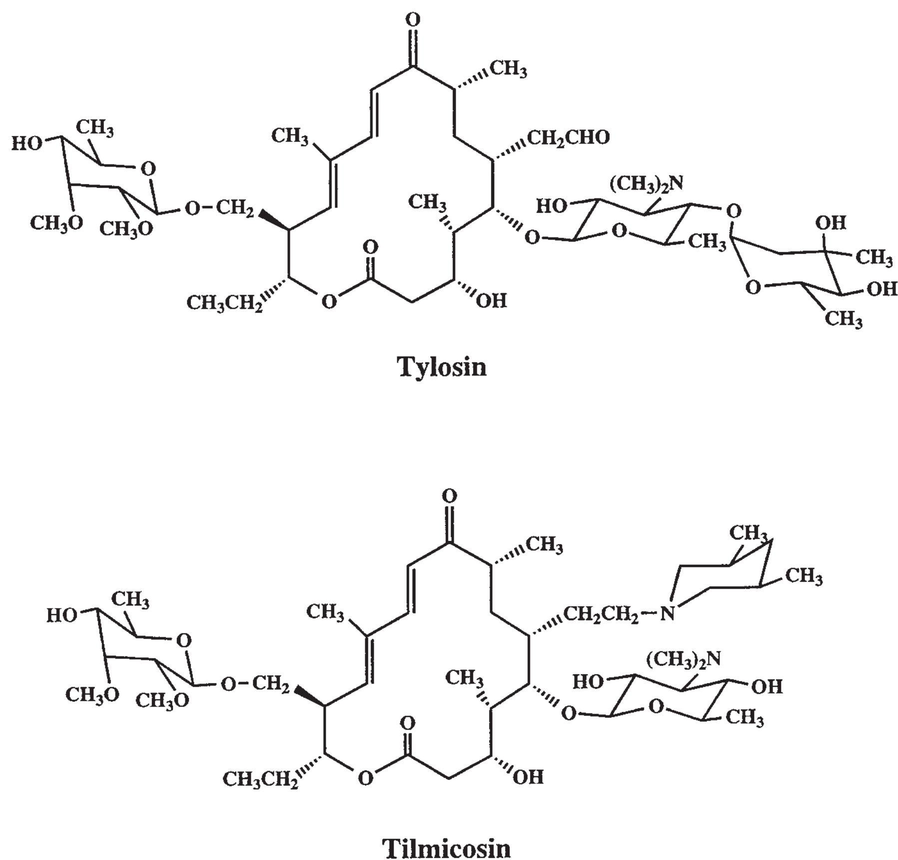

Figure 1 Structures of tylosin and tilmicosin. Tylosin is produced by *Streptomyces fradiae* [18]. Tilmicosin is a semisynthetic derivative of desmycosin [38]. Tylosin and tilmicosin are marketed by the Elanco Animal Health Division of Eli Lilly and Company.

During my first tylosin fermentation production meeting, it became clear that the terminal *O*-methylation of macrocin was the rate-limiting step in tylosin biosynthesis. As the new 'geneticist' at Lilly, I felt compelled to comment on the problem. I told the group that the ultimate solution to poor conversion of macrocin to tylosin would come from recombinant DNA technology. They looked at me with puzzled faces, and someone said: 'What is recombinant DNA technology'? This was 1974, and recombinant DNA technology was in its infancy [34,83]. I decided that development of recombinant DNA technology in streptomycetes should become one of my long-term goals, but I needed to work on efficient mutation and recombination in the short term.

Mutation—the poisson model

At the end of 1974, I attended the Antibiotic Development Division research review. During one of the presentations, data were presented on a mutagenesis protocol for *Streptomyces cinnamonensis*, the producer of monensin. At that time, no one knew what constituted an optimal mutagenic treatment. Should you have '90% kill', or some measurable shift in product potencies within the population of mutagenized cells? What were the best mutagens and mutagenic treatment protocols? There were several ideas but no unifying hypothesis. I formulated a model to optimize mutagenesis based upon the assumption that mutations are distributed randomly within a population of cells. Since the number of mutations per cell should be relatively low, then mutations should be distributed among cells according to the Poisson distribution. Since most mutations that influence antibiotic yields in production strains have negative impacts on yield, the mutational multiplicity could be measured as the fraction of cells that continue to produce control yields (the null fraction of the Poisson distribution). The Poisson model predicts that the optimum mutation frequency, a multiplicity of one mutation per cell influencing product yield, is achieved when the fraction of cells containing no mutations is 37% [6,7]. At this dose, 37% of cells will contain single mutations influencing product yields, and the remaining 26% will contain two or more mutations. This model established a basis to measure the effectiveness of different mutagenic procedures, and has been applied at Lilly since 1975. I published the Poisson model in two book chapters about 12 years after its inception [6,7]. We also characterized mutagenic mechanisms in *S. fradiae*, established that mutation to rifampin resistance and to spectinomycin resistance could be used as surrogate markers to monitor mutagenesis, and carried out comparative studies with different mutagens [6,7,19,20,66,99]. We found in *S. fradiae* that *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) was the only mutagen that consistently gives mutational multiplicities approaching one [6,7]. MNNG has shortcomings in that it induces mutations nearly exclusively

by GC to AT transitions [11]. I have recently proposed a way to broaden the spectrum of induced mutations in actinomycetes by exploiting *mutT* mutations [11].

Genetic recombination

My inclination in 1974 was to develop transduction to recombine beneficial mutations or to eliminate deleterious mutations in streptomycete production strains. My rationale was that transduction with broad-host-range bacteriophages might be applicable to many streptomycetes. I was experienced in bacteriophage genetics, and could imagine other uses for actinophages. We began isolating streptomycete bacteriophages from soil, and identified *Streptomyces griseofuscus* as a good host for phage propagation and assay [36]. *S. fradiae*, the tylosin producer, was not a particularly good host [36,71]. We isolated auxotrophic mutants of *S. fradiae*, and screened for transduction to prototrophy. While these studies were in progress, two articles appeared that demonstrated genetic recombination by protoplast fusion in *Bacillus* species [43,53]. Like transduction, protoplast fusion might be broadly applicable to achieve genetic recombination in streptomycetes. It seemed that protoplast fusion might facilitate recombination over the whole chromosome, and might be applicable to a broader range of actinomycetes. Furthermore, protoplasts capable of regenerating viable cells, as had already been demonstrated with *Streptomyces griseus* and *Streptomyces venezuelae* by Okanishi *et al* [85], might provide a means to develop transformation and recombinant DNA technology in actinomycetes. For these reasons, I immediately initiated studies on protoplast fusion and cell regeneration in *Streptomyces*. Otis Godfrey and I developed protoplast fusion methods, but the patent process postponed the publication of our work until 1978 [4,46], a year after David Hopwood, Merv Bibb, Helen (Wright) Kieser and Stan Cohen published similar work in *Nature* [50]. This was my introduction to the reality of industrial research—the need to establish a patent position on important technology before publication. I was disappointed that we were 'scooped' on publishing the work, but in retrospect, I am pleased that we were 'neck-to-neck' with such an outstanding group of scientists from the John Innes Institute and Stanford University.

Recombinant DNA technology

In 1976, I convinced the management at Lilly that we needed an expert in plasmid biology to develop vectors for recombinant DNA technology in streptomycetes. They agreed, and I recruited Charles Hershberger. To facilitate the anticipated cloning of tylosin genes, Gene Seno and others in my group isolated mutants of *S. fradiae* blocked in tylosin biosynthesis [17,90]. This led to identification of the preferred pathway for tylosin biosynthesis (Figure 2; [22]), verified the rate limiting step in biosynthesis [90,91], and provided strains to produce high levels of novel intermediates and branch products of the tylosin pathway [55,58,59]. The work provided a basis for many patents and for a discovery research program led by Jan Turner and Herb Kirst to modify tylosin intermediates and branch products by bioconversion and by chemical modification. The outcome of this work was the development of tilmicosin (Figure 1), a semisynthetic derivative of desmycosin [38]. Tilmycosin is active against *Actinobacillus pleuropneumoniae*, *Actinomyces pyogenes*, *Mycoplasma hyopneumoniae*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Staphylococcus aureus*, *Streptococcus suis*, and other animal and avian pathogens, and is marketed by Elanco Animal Health (Indianapolis, IN, USA) for the prevention and treatment of respiratory diseases [38,57,81,84,86,102]. The discovery of tilmicosin was an unanticipated spin-off of the *S. fradiae* mutant hunt.

Having established the ability to regenerate viable cells from protoplasts [4,16,65], we began transformation studies in *S. fradiae* in earnest around 1980. By then the John Innes group had demonstrated transformation of *Streptomyces coelicolor* protoplasts [30]. (The Lilly streptomycete vector development program was sidelined in 1978 to accommodate development of recombinant Human Insulin in *Escherichia coli*.) Our first transformation studies demonstrated that *S. fradiae* expresses potent restriction [64]. The bacteriophage plating studies and subsequent mutation studies were in agreement [36,71], and the bacteriophages then proved to be useful tools to rapidly assess the presence of restriction in many different streptomycetes [8,68]. We demonstrated very low efficiency transformation of *S. fradiae* M1 protoplasts [64] after having optimized cell regeneration from protoplasts [16]. *S. fradiae* M1 was deficient in sporulation and was a poor tylosin producer [6], but was the only strain that could be transformed in the early studies. Once the plasmid DNA was passaged through *S. fradiae* M1, it transformed M1 and other *S. fradiae* strains at high efficiency. This confirmed that *S. fradiae* expresses both restriction and modification, and established strain M1 as a useful intermediate host to modify plasmids before introducing them into the highly restricting *tyl* mutants to identify tylosin biosynthetic genes [37,42]. This facilitated cloning and analysis of the tylosin gene cluster at Lilly [18,37,42] and subsequent work in the Cundliffe laboratory [41,44,82,104].

The more general studies on restriction using bacteriophages identified *S. griseofuscus* and *Streptomyces ambofaciens* as relatively non-restricting strains [36]. Since we were able to demonstrate facile protoplast transformation [64], *S. ambofaciens* became an important strain for production of hybrid antibiotics [40,62,87,88]. *S. griseofuscus* also became an important host for vector development and for cloning carbomycin genes [39,63]. My hunch that phages would have broader utility than transduction was borne out.

The middle years (1982–1990)

'*The central dogma of biotechnology: DNA makes RNA, RNA makes protein, and protein makes money*'

Sidney Brenner

In the late 1970s, Lilly licensed recombinant Human Insulin from Genentech. As already mentioned, this had a short-term negative impact on the streptomycete molecular genetics program, since it diverted key resources. However, it had a long-term positive impact on Lilly's committment to recombinant DNA approaches to Discovery and Develop-

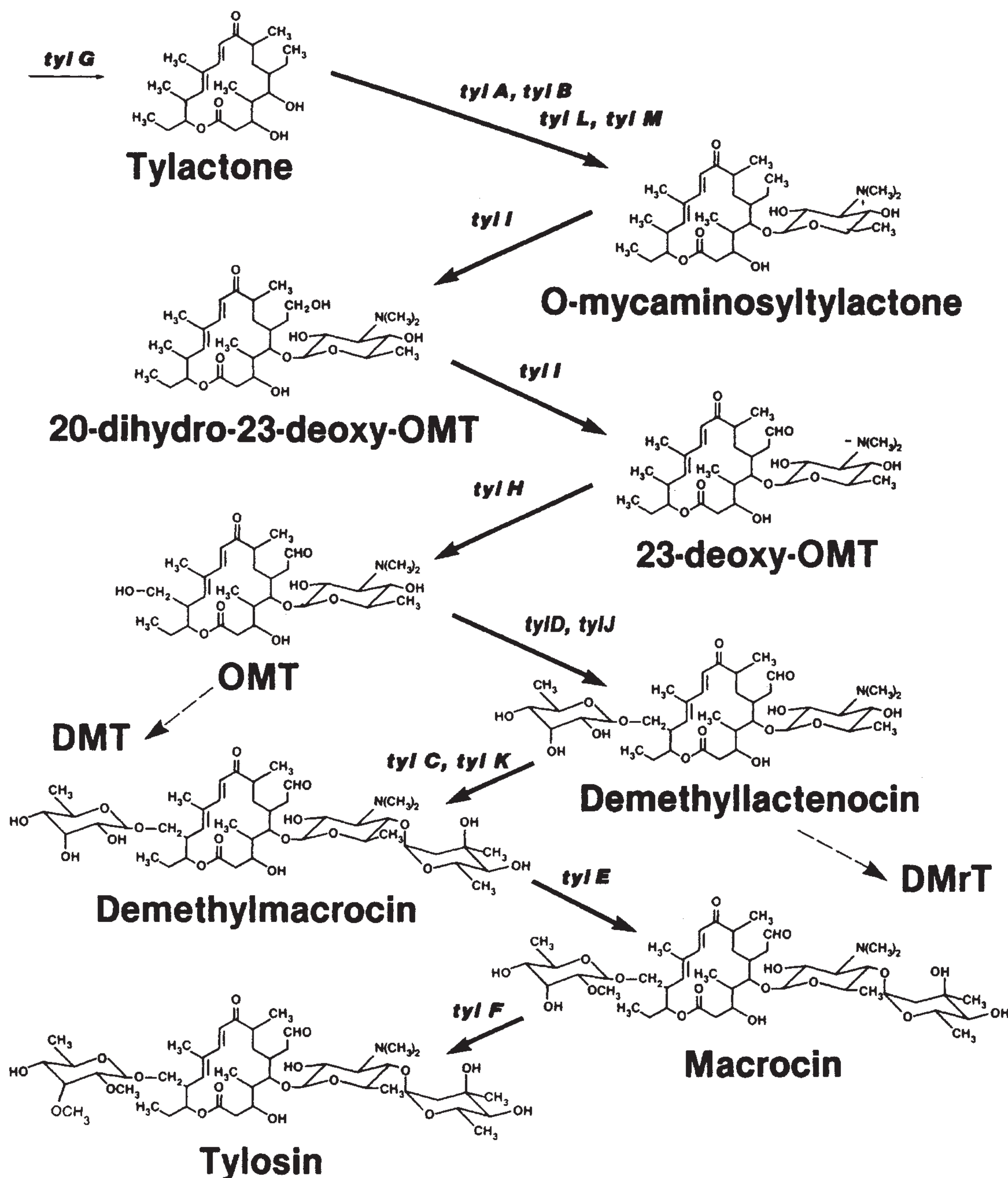

Figure 2 The preferred biosynthetic pathway from tylactone to tylosin [18].

ment. Having been trained in Microbiology and Microbial Genetics, and having been a strong advocate of recombinant DNA technology at Lilly, I was chosen to be Lilly's first Institutional Biosafety Officer, and became a member of the Lilly Institutional Biosafety Committee in 1978. Since the NIH Guidelines on Recombinant DNA Research did not have provisions for scale-up of *E. coli* fermentations beyond 10 liters, I spent much time working with Irving Johnson, Paul Burnett and Max Marsh writing proposals to revise the guidelines. Ultimately, we were successful, and recombinant Human Insulin became the first product of recombinant DNA technology.

As the Molecular and Cell Biology Research group expanded at Lilly during the next 3 years, I assisted Paul Burnett in recruiting. I proposed that recombinant DNA technology might be used to generate novel antibiotics [5], and we established a streptomycete molecular genetics program in the Molecular and Cell Biology Research Division. In 1982, I joined that group to lead the streptomycete research effort, and recruited additional staff to create a Molecular Genetics Department with a broader mission. I agreed to take the group leader position as long as I could maintain a small research program and resign my commission as Institutional Biosafety Officer. From 1982 to 1990, the Molecular Genetics Department developed several different microbial hosts for production of mammalian

proteins, developed molecular genetic tools for manipulation of actinomycetes, and cloned many genes involved in antibiotic biosynthesis. The latter were applied to generate novel antibiotics [23,40,54,62]. During this largely 'tool building' period, my laboratory focused primarily on the development of transformation protocols [64,67,68,72], plasmid transduction [73,75–77], transposition mutagenesis [24,25,49,78,92,93], further characterization of key bacteriophages [47,48], cloning and analysis of macrolide genes [97,98], and production of *S. fradiae* cloning hosts defective in restriction [71,100]. The latter were applied to clone spiramycin biosynthetic genes from *Streptomyces ambofaciens* by heterospecific complementation [88]. Much of this work has been reviewed recently [8,10,15].

The renaissance period (1990–1997)

'*All things at first appear difficult*' Chinese proverb

Spinosad

By 1990, I had shed most administrative duties and had returned essentially full time to research. During the 2-year transition from 1988 to 1990, my lab was expanded to three associate scientists and a postdoctoral student. One project we initiated in 1989 was the development of molecular genetic tools for *Saccharopolyspora spinosa*, the producer of spinosad (Figure 3), a macrolide currently marketed by Dow AgroSciences for insect control. As part of a massive reorganization of Lilly Research Laboratories in 1990, my laboratory group transferred into Natural Products Discovery. This facilitated interactions with Herb Kirst, Jon Mynderse, and Jan Turner, who also worked on the spinosad project. Lilly and Dow Chemical Company formed a joint venture in Plant Sciences about this time, and the spinosad project was transferred to DowElanco. We continued working on the project under contract from DowElanco, but the Lilly resources were greatly reduced. After working with *S. spinosa*, we referred to it as the 'organism from hell' because of its recalcitrance to accept DNA by protoplast transformation. As it turned out, *S. spinosa* expresses potent restriction systems [69]. We found that the restriction barrier could be circumvented by conjugation from *E. coli* [32,74], thus establishing a method to screen a cosmid library for complementation of mutants blocked in spinosad biosynthesis. By using complementation and cosmid homology walking, we and collaborators at DowElanco were able to clone and sequence the 80-kb segment of the chromosome that encodes most of the spinosyn biosynthetic genes [103]. These and other genes are being used in a strain improvement program at Dow AgroSciences.

Daptomycin

My group transferred into the Infectious Disease Discovery Research Division in 1992. We continued developing transposons for streptomycetes, and applied them to clone the daptomycin biosynthetic genes [79,80]. Our goal was to modify the daptomycin peptide synthetase to incorporate different amino acids into the 13-member ring. Daptomycin (Figure 4) is a potent antibiotic with bactericidal activity against Gram-positive pathogens, including methycillin-resistant *S. aureus*, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae* [9]. We cloned the genes for biosynthesis of daptomycin and A54145 (a related lipopeptide) [26,80]; developed the *rpsL* system for direct selection of double crossovers [52]; and

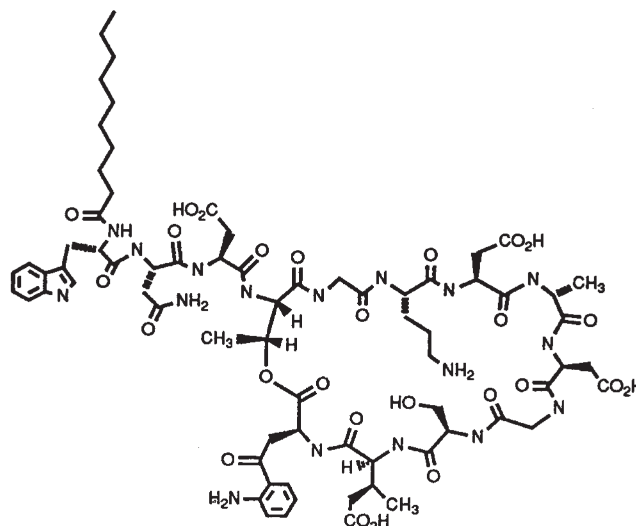

Figure 4 Structure of daptomycin. Daptomycin is produced by *Streptomyces roseosporus* [9], and is in clinical trials sponsored by Cubist Pharmaceuticals.

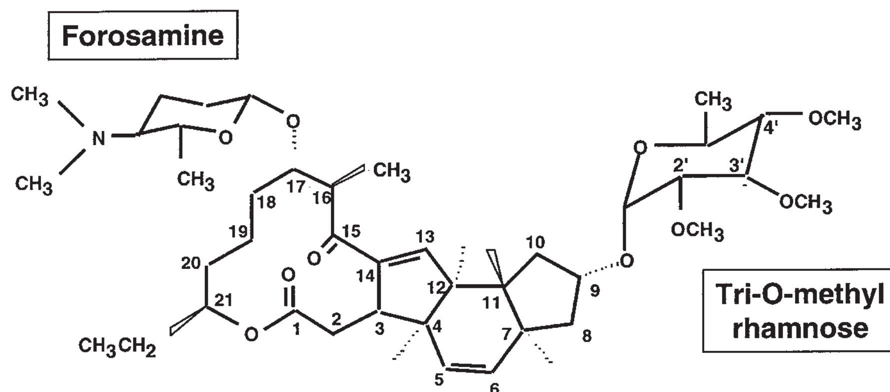

Figure 3 Structure of spinosyn A. Spinosyns are produced by *Saccharopolyspora spinosa* [74,103], and marketed by Dow AgroSciences as insect control agents.

isolated mutants that expressed enhanced homeologous recombination to facilitate *in vivo* recombination between daptomycin and A54145 genes [51]. Unfortunately, daptomycin was withdrawn from clinical trials, and Lilly later dropped all work on derivatives of daptomycin. This decision precluded exploiting the successes of the project, and we discontinued the remaining limited work on making hybrid peptide synthetases when my postdoctoral student, Tom Hosted, took a job at Schering Plough Research Institute in 1996. However, the daptomycin story is not yet finished. I presented the daptomycin work in a seminar at Cubist Pharmaceuticals late in 1996. In the introduction, I reviewed the attributes of daptomycin, which I summarized in a review article [9]. Cubist took an interest in daptomycin, and licensed it from Lilly. Under Cubist sponsorship, daptomycin is in Phase II clinical trials for the treatment of bacteremia and in phase III trials for skin and soft tissue infections caused by Gram-positive pathogens.

Glycopeptides

The Infectious Disease Discovery Research Division became interested in developing a second generation glycopeptide around the time that clinical trials on daptomycin were discontinued. They were developing derivatives of chloroeremomycin (A82846B), a glycopeptide related to vancomycin (Figure 5; [35]). In early 1994, I proposed a molecular genetics approach to prepare hybrid glycopeptides for chemical modification. The clinically useful glycopeptides, vancomycin and teicoplanin (Figure 5), contain different heptapeptide core structures. I proposed to engineer *Streptomyces toyocaensis*, the producer of the non-glycosylated teicoplanin heptapeptide, to produce a molecule containing the sugars normally present on chloroeremomycin. This would generate a novel structure that could be chemically modified by reductive alkylation in the same manner as LY333328 [35], a potent glycopeptide antibiotic in clinical trials that is active against Gram-positive pathogens, including vancomycin-resistant enterococci [1,45,56,89]. Since the molecular genetic approach required some lead time to develop the gene transfer system in *S. toyocaensis*, to clone the chloroeremomycin and vancomycin genes, to express the glycosyltransferase genes in *E. coli* and *S. toyocaensis*, etc, we sought assurances that the Infectious Disease Strategy Group would be interested in pursuing novel glycopeptides for at least 3 years. There was no point in starting the project if the timelines were shorter. Having received an endorsement of the project and assurances of a 3-year minimal committment, we initiated the project. In the first 2 years, we developed a gene transfer system and chromosomal integration in *S. toyocaensis* [70], using vectors that had been developed by others in the Lilly actinomycete molecular genetics group [32,60,61]. We also cloned the chloroeremomycin gene cluster from *Amycolatopsis orientalis*; identified the glycosyltransferase genes from the vancomycin- and chloroeremomycin-producing strains of *A. orientalis*; expressed the glycosyltransferase genes in *E. coli* and *S. toyocaensis*; and produced a novel monoglycosylated derivative of A47934 (Figure 5; [95,96]). This demonstrated the feasibility of the approach, and provided the substrate for the next two glycosylations. The Vice President who gave the assurances on the project timelines resigned from Lilly in 1996. The interim management team discontinued the glycopeptide, macrolide, and β-lactam programs, as well as a number of antifungal and antiviral programs, much to the astonishment of the scientists in the Division. I am assured by colleagues in the industry that such acts occur periodically in all big pharmaceutical companies, and that they are usually associated with the changing of the guard.

We patented the five glycosyltransferase genes cloned from the vancomycin- and chloroeremomycin-producing strains of *A. orientalis*, and arranged to have the cosmids containing the chloroeremomycin biosynthetic genes sent to Dudley Williams. His group completed the DNA sequencing of this important set of genes [101].

Streptococcus pneumoniae genomics

When I joined the Infectious Disease Discovery Research Division in 1992, there were several antibacterial research projects, but no research engine to generate new projects. In 1994, I proposed a modest genomics project, focused on *S. pneumoniae*, to generate new targets for antibiotic development. Paul Rosteck and I devised a DNA sequence sampling approach to identify genes present in *S. pneumoniae* and to facilitate gene disruption to identify potential lethal targets. Much of my resources previously dedicated to daptomycin, spinosad, and glycopeptide molecular genetic projects were diverted into *S. pneumoniae* genomics. We carried out DNA sequencing and developed a robust gene disruption technique that employed conjugation from *E. coli* [28,29]. We identified many potential lethal targets for antibiotic intervention, and bacterial genomics became the antibacterial research engine in the Infectious Disease Discovery Research program at Lilly.

Other transposon applications

My group collaborated with Steve Queener's group in Antibiotic Development to demonstrate that transposons could be used to clone neutral genomic sites for the insertion of cloned tylosin biosynthetic genes in *S. fradiae*. We used this methodology to demonstrate improved tylosin yield in a production strain containing an extra copy of the *tylF* (macrocin-*O*-methyltransferase) gene [27,94]. My prediction made at the tylosin production meeting in 1974 was realized.

Back to the future (1997–1999)

'*Go where the puck is going, not to where it is*'
Wayne Gretzky

Having witnessed the premature death of the daptomycin and glycopeptide projects, the earlier discontinuation of macrolide and β-lactam molecular genetics programs, and the transfer of the spinosad project to DowElanco, it was time for me to move to an environment more supportive of actinomycete secondary metabolite research. I took a sabbatical leave from Lilly in 1997, and rekindled my interest in spinosad and natural products discovery at DowElanco (now Dow AgroSciences). I retired from Lilly in 1998, and joined Dow AgroSciences as resident consultant.

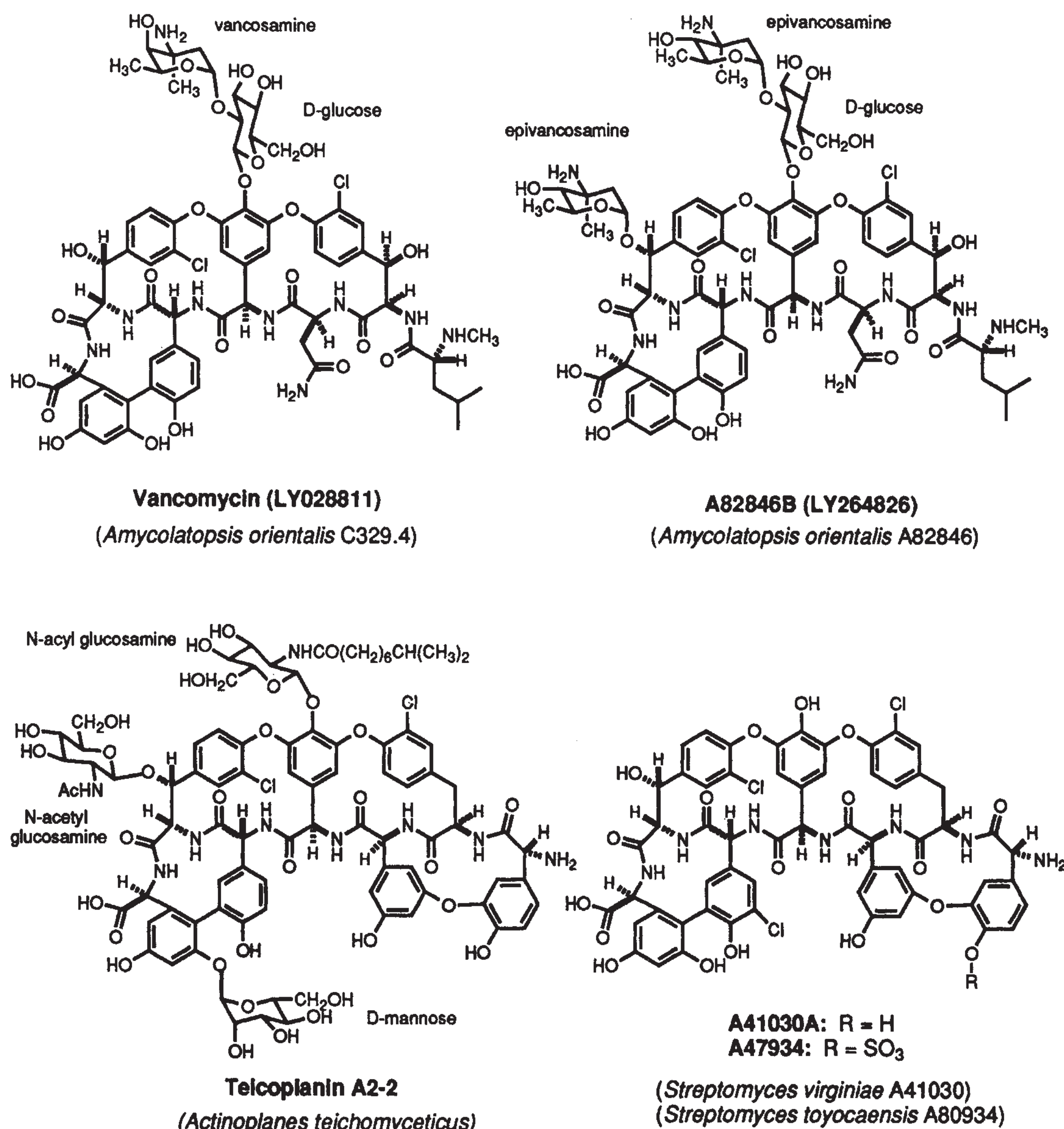

Figure 5 Structures of glycopeptide antibiotics. The producing actinomycetes are listed below the structures.

With the visionary support of Dow AgroSciences management, Clegg Waldron and I established a molecular genetics group to work on spinosad yield improvement. I founded CognoGen Biotechnology Consulting in 1999, and am working with different companies in the areas of combinatorial biosynthesis, antibiotic development, natural products discovery research, and microbial genomics. The golden years of actinomycete molecular genetic applications to yield enhancement and to discovery of novel secondary metabolites are now just beginning. The many years of molecular genetic tool building and cloning of secondary metabolite genes will likely pay big dividends to companies poised to exploit the wide-ranging biological activities and rich chemical diversity inherent in actinomycete secondary metabolites.

Epilog

So what about the title 'Sweet Home Actinomycetes'? I was sitting in a pub with two of my brothers in Seattle after the SIM Annual Meeting in 1988, sipping a product of ancient biotechnology. A Canadian blues band played 'Sweet Home Saskatchewan'. It was a great rendition of 'Sweet Home Chicago'. But anyone who enjoys blues knows that 'Sweet Home Chicago' was derived from the Big Joe Williams and JD Short song 'Sweet Old Kokomo', which was probably derived from some other more primitive country blues song. All three renditions speak to two themes: successful endeavors build on the best ideas of others in your field; and there are places that just feel like home. I have been privileged to experience both in my studies on actinomycetes.

Acknowledgements

I thank SIM and MDS Panlabs for the honor of presenting the MDS Panlabs lecture. I dedicate the lecture to the memory of my father, Henry John Baltz, who died 25 years ago. He didn't spend a lot of time talking, but when he did, it was usually worth listening. He said things like: 'Save your money for college'; 'If it's worth doing, it's worth doing right'; and 'Do something interesting with your life'. Those simple but profound words of guidance have served me well over the years. I also thank Mel Novak, my high school basketball coach, who taught me that success in basketball (or in any endeavor) can be attributed to 10% raw talent and 90% hard work. (I didn't actually believe him at the time, but have come around to his thinking.) I thank Jan Drake for giving me my start in science; Larry Day, RQ Thompson, Dave Dennen, Paul Burnett, Wayne Millar, John Whitney, Irving Johnson, Neal Pettinga, Barry Eisenstein, and Carlos Lopez for supporting my work at Lilly; Len Smith, Bill Kleschick, Dick Tobey, and Cliff Gerwick for supporting my work at Dow AgroSciences; Sam Kaplan, David Hopwood, and Rich Losick for good advice along the way; Patti Matsushima Treadway, Pat Solenberg, Margaret McHenney, Jill Stonesifer Gonzales, Karen Cox, Don Hahn, Tom Hosted, Tom Ingolia, Jim Miller, Paul Skatrud, Paul Rosteck, Jan Turner, Herb Kirst, Steve Queener, Don Merlo, Clegg Waldron, and Eric Cundliffe, who have been exceptional collaborators and colleagues; Chuck Hershberger, Stu Kustoss, Nagaraja Rao, Brigitte Schoner and Gene Seno for important contributions to the actinomycete molecular genetics program at Lilly; and the broader community of actinomycete scientists who do good science, share knowledge and resources, and enjoy each other's company. This community has provided a 'sweet home' that I could not have imagined when I moved from bacteriophage T4 to take the 'less traveled' actinomycete road 25 years ago.

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