MOLECULAR BIOLOGY OF STREPTOMYCETES

Organizers: Mervyn Bibb, Richard Losick and Dean Taylor

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Plenary Sessions Page

Poster Sessions

From Primary to Secondary Metabolism-I

CC 001 THE STRINGENT RESPONSE IN Streptomyces coelicolor A3(2). Mervyn Bibb and Eckhard Strauch, John Innes Institute, Norwich NR4 7UH, U.K.
The stringent response has been implicated as one of the triggers for antibiotic production and sporulation in Streptomyces. We have initiated a study of this phenomenon in the genetically well defined strain S. coelicolor A3(2). We can elicit the stringent response either by nutritional shift-down or by addition of serine hydroxamate. Both treatments result in accumulation of ppGpp, depletion of GTP and a reduction in rRNA transcription. The effect of inducing the stringent response on the onset of antibiotic production in this species is being assessed. If an effect can be established, the mechanisms leading to the activation of a number of regulatory genes, some pleiotropic, whose expression is required for both antibiotic production and differentiation will be analysed and described.

CC 002 REGULATION OF GLUTAMINE SYNTHETASE IN STREPTOMYCES COELICOLOR, Susan H. Fisher, Lewis V. Wray and Mari Atkinson, Department of Microbiology, Boston, University School of Medicine, Boston, MA 02118
Glutamine synthetase (GS) expression in S. coelicolor is transcriptionally regulated by nitrogen availability. In addition, its enzymatic activity can be inactivated by adenylylation of the GS protein. In order to genetically identify the S. coelicolor glnA gene and gene products involved in its regulation, glutamine requiring (Gln^-) mutants were sought. Six Gln^- mutants which require glutamine for growth on all medium have been isolated. The phenotype of these mutants is remarkably similar. GS levels were reduced 25- to 100-fold in extracts of the Gln^- mutants compared to wild-type cells. The lowered GS levels in the Gln^- extracts is not due to adenylylation because GS activity in the Gln^- extracts was unaltered by incubation with snake venom phosphodiesterase. No glnA transcripts could be detected in primer extension experiments using RNA isolated from the Gln^- mutants. All six gln mutations mapped adjacent to adeA. A 1.6 kb DNA fragment that complements all the Gln^- mutants has been cloned using low copy Streptomyces plasmids and is being sequenced. The complementation data indicates that two genes are encoded by this DNA fragment. Since the cloned complementing DNA does not cross hybridize with S. coelicolor glnA DNA, these mutants appear to be deficient in a gene required for glnA transcription (GlnR). Indeed, transcription of the glnA gene in S. coelicolor is likely to require a positive factor since the nucleotide sequence of the glnA promoter has significant homology with the -10, but not with -35 consensus sequence for Streptomyces vegetative promoters. Hybridization of the wild-type and mutant restricted chromosomal DNA with the cloned GlnR DNA showed that the glnR loci in three of the Gln^- mutants contained either a site specific insertion sequence or a chromosomal rearrangement. This suggests that the DNA fragment which complements the glnR mutations is the wild-type GlnR DNA and not a DNA fragment that suppresses the Gln^- phenotype of these mutants. Surprisingly, no glnA mutations have been isolated. It is possible that glnA mutations are lethal in S. coelicolor or that multiple GS enzymes are expressed in S. coelicolor.

CC 003 THE INTERACTION OF PRIMARY AND SECONDARY METABOLISM: PROLINE METABOLISM AND UNDECYLPRODIGIOSIN ANABOLISM IN Streptomyces coelicolor A3(2), David A. Hodgson, Derek Hood and Uthaya Swoboda, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, UNITED KINGDOM.

We set out to investigate the biosynthesis, uptake and degradation of proline in Streptomyces coelicolor A3(2). We chose this amino acid because: i) in general, so little is known about amino acid metabolism in streptomycetes; and ii) in particular, proline is a precursor of Undecylprodigiosin (the red/yellow pigment), one of the seven secondary metabolites of this streptomycete.

Proline Uptake and Breakdown. Using a number of proline analogues we have isolated a variety of mutants of Stm. coelicolor A3(2) unable to transport proline. Some of these mutants are also unable to breakdown proline. Interestingly, these mutants incapable of transport or transport and breakdown of proline overproduced Undecylprodigiosin. Our interpretation of this result is that if excess proline can not be exported or degraded it is pushed into the sink of secondary metabolism. If this hypothesis is correct, we would predict that proline synthesis is not feedback regulated.

Proline Biosynthesis. We have obtained a clone of the Stm. coelicolor A3(2) proline biosynthetic genes. Subcloning of the DNA has revealed the Stm. coelicolor A3(2) and Stm. lividans pro mutants available to us fall into two complementation groups. We have recently finished sequencing the 4 Kb of DNA that encode the two complementing regions. If our suggestion that proline synthesis is not subject to feedback regulation is correct, we might expect that transfer of the pro^+ genes onto a multicopy vector might stimulate Undecylprodigiosin production. A strain containing such a construct does, indeed, overproduce Undecylprodigiosin.

CC 004 POST-TRANSLATIONAL REGULATION OF HISTIDASE ACTIVITY IN STREPTOMYCES, Kathleen E. Kendrick, Department of Microbiology, Ohio State University, Columbus, OH 43210

The amino acid L-histidine serves as an efficient nitrogen source for Streptomyces griseus. None of the histidine utilization (hut) enzymes is active in cells grown in the absence of L-histidine, and expression of the hut enzymes is not subject to nitrogen regulation. To learn the details of the regulation of the hut system, we have characterized histidase activity in the wild-type strain and Hut^- mutants of S. griseus. The curved reaction kinetics of histidase in extracts suggested that histidase undergoes activation in vitro. Subsequent experiments using a mutant that was impaired in its ability to activate histidase established not only that activation of histidase occurred in vivo, but also that the activation of histidase was reversible and effected by at least one protein (AF) that could be replaced by snake venom phosphodiesterase in vitro. Although the lack of histidase activity in extracts from the wild-type strain grown in the absence of histidine mirrored the inducible hut systems in other bacteria, evidence clearly shows that histidase is synthesized constitutively by S. griseus and is maintained in an inactive form until L-histidine or urocanate (one of the products of histidase activity) is added to the medium. Our working model of regulation of histidase activity invokes a cascade of activation: the presence of L-histidine or urocanate in the environment causes activation of AF, which in turn activates histidase by a mechanism that involves hydrolysis of a nucleotidyl moiety; depletion of the supply of L-histidine or urocanate leads to inactivation of AF and, thus, of histidase. AF appears to be a protein comprising two dissimilar subunits, one of which is independently catalytic and the other of which is required for activation of histidase. Mutants that are defective in the activation or inactivation of histidase are also aberrant in their ability to synthesize the antibiotics streptomycin and cycloheximide, suggesting that the cascading system that regulates histidase activity may also be involved in the control of antibiotic synthesis.

CC 005 THE PHYSIOLOGICAL SWITCH TOWARDS SECONDARY METABOLISM, Stephen G. Oliver, Manchester Biotechnology Centre, UMIST, PO Box 88, Manchester M60 1QD, England. A complete understanding of the control of the switch from primary to secondary metabolism in Streptomyces bacteria requires an integration of molecular genetic and physiological data. At UMIST, we have aimed to build on the genetic foundations established for Streptomyces coelicolor by developing techniques which allow the study of the physiology of antibiotic production under defined conditions. A chemically defined medium has been formulated which uses a charged polymer to promote dispersed growth and so permit the study of physiologically homogeneous cultures. This medium has been used to study the production of undecylprodigiosin, actinorhodin and methylenomycin in batch culture and to demonstrate that the synthesis of these three products is separately controlled. Our studies have also permitted the continuous production of these antibodies in chemostat culture and have demonstrated fundamental differences between the control of the production of growth-associated and secondary products. Continuous growth can lead to genetic instability by selecting for mutant organisms which no longer produce antibiotics. The selective advantage which accrues to such mutants varies according to the limiting nutrient employed, so permitting the physiological control of instability. Genetic variation within populations undergoing continuous growth may be exploited in the isolation of over-producing mutants by the use of computer-controlled selection regimes.

From Primary to Secondary Metabolism-II

CC 006 LOCI INVOLVED IN REGULATION OF ANTIBIOTIC SYNTHESIS, Wendy Champness, Perry Riggle and Trifon Adamidis, Genetics Program and Department of Microbiology, Michigan State U. East Lansing, MI 48824

Recently we have searched for S. coelicolor mutations which globally block antibiotic synthesis without affecting sporulation. Our initial search led to the discovery of the absA locus. Mutations in this locus prevent synthesis of actinorhodin, undecylprodigiosin, methylenomycin and calcium-dependent antibiotic, without affecting sporulation or growth rate. The Abs$^-$ phenotype in absA mutants does not vary with nutritional condidions. The absA locus is distinct from two other loci reported to be involved in antibiotic synthesis- afsB and afsR. In the course of testing the possible identity of afsR and the absA locus, we found that multiple cloned copies of afsR can bypass the block to antibiotic synthesis in absA mutants.

Because the initial search did not produce any candidates for afsB or afsR mutants, we conducted further searches, resulting in identification of an additional locus, which we have named absB. The Abs$^-$ phenotype in absB mutants is somewhat leakier than in absA mutants. Multiple copies of afsR also bypass the absB phenotype.

CC 007 ANTIBIOTIC BIOSYNTHESIS IN A bldA MUTANT OF Streptomyces coelicolor. Ellen P. Guthrie*, David A. Hopwood, and Keith F. Chater, John Innes Institute, Norwich NR4 7UH, U.K., * present address, New England Biolabs, 32 Tozer Road, Beverly, MA 01915

Streptomyces coelicolor bldA mutants, which are defective in a tRNA for a rare codon TTA, normally produce no antibiotics nor, on certain carbon sources, any aerial mycelium. Certain physiological and genetic changes have been found to give partial relief of the pleiotropic effect on antibiotic biosynthesis, allowing these bldA mutants to produce undecylprodigiosin, one of the pigmented antibiotics normally produced in the wild type S. coelicolor. These results indicate that the red structural genes, which encode undecylprodigiosin biosynthesis, do not contain the codon TTA, which depends on the bldA-specified tRNA for translation, and therefore that bldA acts indirectly through a regulatory cascade. Transcriptional fusions to a xylE reporter gene suggests that for at least one red gene the effects described here are mediated at the transcription level, implying that the bldA tRNA is involved in the synthesis of a protein that either directly or indirectly influences red gene transcription.

CC 008 REGULATION OF SECONDARY METABOLISM AND MORPHOGENESIS IN STREPTOMYCES, Sueharu Horinouchi and Teruhiko Beppu, Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

I. A global regulatory gene afsR (afsB), from Streptomyces coelicolor A3(2)

The afsB gene of Streptomyces coelicolor A3(2) which is required in this organism for the biosynthesis of A-factor and the pigmented antibiotics actinorhodin and undecylprodigiosin. We have found that the AfsB protein contains two regions which resemle conserved domains of known DNA-binding proteins and that it stimulates transcription of the actinorhodin biosynthetic pathway. we have also found that a region (afsC) upstream of afsB enhances the stimulatory function of afsB. To our surprise, nucleotide sequencing and subsequent experiments revealed that afsC and afsB comprised actually a single open reading frame. Furthermore, the amino acid sequence derived from the afsC region contains two consensus sequences for ATP-binding. Preliminary experiments suggested that the AfsBC protein was autophosphorylated.

II. The A-factor-receotor protein of Streptomyces griseus is a repressor-type regulator

By using 3H-A-factor, a binding protein for A-factor was detected in the cytoplasmic fraction of this organism. The binding protein had an apparent molecular weight of approximately 26,000, as determined by gel filtration chromatography. Scatchard analysis suggested that A-factor bound the protein in the molar ratio of 1:1 with a binding constant, K_d, of 0.7 nM. The number of the binding protein was roughly estimated to be 37 per genome. The mutants deficient in the A-factor-binding protein derived from an A-factor-negative mutant of S. griseus began to produce streptomycin and spores in the absence of A-factor. These data strongly suggest that the A-factor-binding protein is a repressor type regulator.

III. A-factor-dependent promoters in the streptomycin biosynthetic cluster

The streptomycin (Sm) resistance gene encoding Sm-6-phosphotransferase is located in the Sm biosynthetic cluster. Piepersberg's group reported that the three promoters are all inactive in the absence of A-factor. By using an extremely heat-stable malate dehydrogenase gene as a reporter, we are examining A-factor-dependence of the promoters in part of the cloned cluster that contains at least three transcriptional units.

Antibiotic Biosynthesis-I

CC 009 PROPERTIES OF GENES AND ENZYMES FOR THE BIOSYNTHESIS OF ANTITUMOR ANTHRACYCLINE ANTIBIOTICS, C. Richard Hutchinson*, Kim J. Engwall*, Patrick G. Guilfoile*, Fumio Kato*, Haideh Motamedi*†, Hiroshi Nakayama*, Sharee L. Otten*, Richard G. Summers*, Evelyn Wendt-Pienkowski*, Bill L. Wessel*, Randi L. Rubint, Stanley L. Streichert, Mervyn J. Bibb§, Hugo Gramajo§ and Janet White§, *School of Pharmacy and Department of Bacteriology, University of Wisconsin, Madison, WI 53706; †Department of Infectious Disease Research, Merck Sharpe & Dohme Research Laboratories, P. O. Box 2000, Rahway, NJ 07065; §John Innes Institute and AFRC Institute of Plant Science, Norwich NR4 7UH, UK.

Studies of the gene clusters governing the biosynthesis of tetracenomycin C by *Streptomyces glaucescens* and daunorubicin by *Streptomyces peucetius* have led to interesting insights into the organization and function of polyketide synthases and the regulation of secondary metabolism in these bacteria. The polyketide synthase that catalyzes the biosynthesis of tetracenomycin F1, the earliest known intermediate of the tetracenomycin C pathway, appears to consist of at least five individual proteins. Some of these enzymes can be assigned functions analogous to the enzymes of fatty acid biosynthesis (acyl carrier protein, β-ketoacyl synthase), two of which have been purified from *S. glaucescens*, while others are unique to polyketide biosynthesis (tetracenomycin F1 cyclase). Transcription of the *tcmIa* and *tcmVI orf*s that encode these enzymes as polycistronic mRNAs appears to depend on a promoter just upstream of *tcmVI* and may be regulated by the adjacent *tcmIb* or *tcmIc* genes. Complementation of *tcmIa* and *tcmId* but not *tcmVI* mutations was obtained with a 3.8 kb DNA fragment cloned from the salinomycin-producing *Streptomyces albus* on the basis of hybridization to the *tcmIa* and *Streptomyces coelicolor actI* and *actIII* genes. Four regions of DNA hybridizing to the *tcmIa* and *actI* genes also reside in *S. peucetius*; region IV contains most of the daunorubicin production genes and cosmid clones from this and the three other regions modulate the production of ε-rhodomycinone (an intermediate of the daunorubicin pathway) and daunorubicin by *S. peucetius* transformants. On the basis of the considerable overproduction of these metabolites by such clones and their derived subclones, two ≈2 kb *Bam*HI fragments separated by 12.6 kb have been identified in the daunorubicin cluster. From their ability to complement the mutation(s) blocking daunorubicin production by *S. peucetius* H6101 or to stimulate actinorhodin production by *Streptomyces lividans*, we believe they contain genes that regulate secondary metabolism in *S. peucetius*. The regions adjacent to one of these fragments appear to contain genes that confer daunorubicin self-resistance and that stimulate sporulation in wild-type *S. peucetius* transformants.

CC 010 GENETICS OF ERYTHROMYCIN SYNTHESIS IN Saccharopolyspora erythraea, Leonard Katz, Michael Staver, James Tuan, David Brown and Stefano Donadio, Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60064

Erythromycin [Er] is a macrolide antibiotic produced in Saccharopolyspora erythraea through a complex biochemical pathway that involves (1) formation of the 14-membered macrolactone ring 6-deoxyerythronolide B, [6dEB] from the condensation of propionyl CoA and 6 methylmalonyl CoA residues, (2) synthesis of the sugars D-mycarose and desosamine and their attachment to the ring to form erythromycin D [ErD] and (3) hydroxylation and O-methylation of ErD to form ErA. Genes involved in the synthesis of the sugars or their attachment to the ring are designated eryB, eryC and eryD and have been located on the chromosome clustered about ermE, the gene that determines MLS resistance in Sac. erythraea. eryG, which determines the O-methyltransferase involved in the final step of ErA synthesis, is also located in this region and has been cloned and expressed in E. coli. Genes that are involved in the synthesis of 6dEB are designated eryA and appear to be clustered in a 30 kb segment of the chromosome beginning 12 kb downstream of ermE. Nucleotide sequence analysis of this segment has revealed the presence of several open reading frames. Deduced amino acid sequences of the corresponding polypeptides contain domains that resemble sequences of polypeptides involved in fatty acid synthesis: acyltransferase, β-ketosynthase, reductase and ACP. Most of the ORFs encode a polypeptide with a single putative function but some of the polypeptides appear to serve two enzymic functions. The eryA ORFs are organized in a series of repeated modules of approximately 5 kb with each module containing an acyltransferase, ACP, β-ketosynthase and reductase. The complexity of the structure of 6dEB relative to that of fatty acids and simple polyketides is likely reflected in the greater number of genes involved in its synthesis. We sugggest that, in the synthesis of 6dEB, each module in eryA determines the enzymic activities required for the condensation and processing of each of the methylmalonyl CoA residues in the the growing acyl chain.

CC 011 MOLECULAR GENETICS OF POLYKETIDE ANTIBIOTIC BIOSYNTHESIS IN STREPTOMYCES.

David H. Sherman, Maureen J. Bibb, and David A. Hopwood, John Innes Institute, Norwich NR4 7UH, U.K.

As part of a collaborative effort to try to understand the basis of the programming of polyketide synthases (PKSs), our work has focussed on a 6.5 kb region of DNA from S. violaceoruber which contains PKS genes for production of the benzoisochromane quinone moiety of the antibiotic, granaticin(1). DNA sequencing has revealed six open reading frames (ORFs) which are contained in two divergently transcribed groups. Comparison of the individual deduced protein sequences coded by these ORFs has revealed striking similarities with fatty acid synthase condensing enzymes, acyl carrier proteins and oxidoreductases. Additional data suggest that one of the granaticin PKS ORFs encodes a dehydrase/cyclase which may determine the ultimate polyketide structural type. Comparisons of the organization of the granaticin PKS genes with that of PKS genes involved in the biosynthesis of several other polyketides reveals a highly conserved gene arrangement.

In order to obtain material for biochemical studies we are attempting to over-express in E. coli specific Streptomyces PKS genes. Using an inducible T7 RNA polymerase-based system(2), we have been able to express significant levels of the granaticin PKS acyl carrier protein (ACP). This work will provide the basis for future studies on the possible role of the ACP as a control element in polyketide antibiotic biosynthesis.

1. Sherman, D.H., F. Malpartida, M.J. Bibb, H.M. Kieser, M.J. Bibb and D.A. Hopwood. (1989) EMBO J., 8, 2717-2725.
2. Tabor, S., and C.C. Richardson. (1985) Proc. Nat'l. Acad. Sci. USA, 82, 1074-1078.

Antibiotic Biosynthesis-II

CC 012 MOLECULAR GENETICS OF OXYTETRACYCLINE PRODUCTION BY Streptomyces rimosus. Iain S Hunter, Deirdre Doyle and Kenneth J McDowall, Robertson Institute for Biotechnology, University of Glasgow, Church Street, Glasgow G11 5JS, UK.

Oxytetracycline is a polyketide antibiotic, composed entirely of acetate units. Using resistance to the drug as a selectable marker (1), two closely-linked resistance genes and four biosynthetic genes were cloned (2). Subsequently, all of the biosynthetic genes were shown to be encoded between the two resistance genes (3). When this DNA was introduced into S. lividans, it made oxytetracycline.

On its own, this DNA is not sufficient to encode gene products which will synthesise oxytetracycline. Unusual flavin cofactor(s) are required, which appear to be ubiquitous within streptomycetes. Genes for their biosynthesis map elsewhere on the chromosome.

By functional analysis and DNA sequence comparisons, the resistance genes encode a rimosomal modification (otrA - possibly an alternative EF-Tu) and export protein (otrB, also called tet347 [4]).

The polyketide assembly genes are transcribed divergently from otrB. Some of the genes which add peripheral functional groups are transcribed tandemly with otrA.

Transcripts (which are subject to regulation) diverging from the otrA promoter have been mapped. The data suggest a plausible mechanism for control of expression of production genes.

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2. Butler, M.J. et al. (1989). Mol. Gen. Genet 215, 231-238.
3. Binnie, C. et al. (1989). J. of Bacteriol. 171 (2), 887-895.
4. Reynes, J-P. et al. (1988). J. of Gen. Mic. 134, 585-98.

CC 013 THE GENES FOR AVERMECTIN BIOSYNTHESIS ARE CLUSTERED, Douglas J. MacNeil, Keith M. Gewain, Carolyn L. Ruby, Tanya MacNeil and Patti H. Gibbons, Department of Microbial Chemotherapeutics and Molecular Genetics, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065

Streptomyces avermitilis produces a series of 8 potent anthelmintic compounds called avermectins. The avermectins are polyketide, macrocyclic lactone compounds containing an oleandrose disaccharide. Previously, two nonadjacent clones which complemented *S. avermitilis* mutants defective in modifying the avermectin ring structure by O-methylation and glycosylation were isolated. We have now located a large cluster of genes involved in avermectin biosynthesis. Over 140 kb of DNA containing the genes for avermectin biosynthesis was isolated from a cosmid library by a series of chromosome walking experiments from the region involved in glycosylation. These cosmids overlapped the previously cloned genes for modification of avermectin. In one region the structure of the cosmid clones was very heterogeneous, suggesting that rearrangements different from the chromosomal order occurred in this region. This region contained several members of two groups of related sequences found in *S. avermitilis*. The chromosomal arrangement in this region was determined using a unique in vivo cloning procedure. Many genes for avermectin biosynthesis were localized by subcloning and complementation analysis, as well as by transposon mutagenesis of the cloned regions. In addition to identifying several genes involved in avermectin macrocyclic lactone ring formation, the genes for furan ring closure, and dehydration were located. The genes for oleandrose synthesis and addition to avermectin aglycone are clustered in a 12 kb region located at one end of the avermectin gene cluster.

CC 014 MOLECULAR CHARACTERIZATION OF THE BETA-LACTAM BIOSYNTHETIC PATHWAY IN *Streptomyces clavuligerus*: SEQUENCE, EXPRESSION, AND TRANSCRIPTION STUDIES. James R. Miller*, Steven Kovacevic, and Matt Tobin. Department of Molecular Genetics, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

Four out of six genes coding for enzymatic steps of the beta-lactam biosynthetic pathway leading to cephalosporin C have been characterized at the molecular level[1-3]. In *Streptomyces clavuligerus*, all of the genes may be linked and at least two genes are coordinately expressed as an operon. Our studies show that Isopenicillin N isomerase ("epimerase") and Deacetoxycephalosporin C synthetase ("expandase") are coded for on a single transcript. Studies from other laboratories indicate that ACV synthetase, the first enzyme in the pathway, is closely linked to Isopenicillin N synthetase ("cyclase"), the second. The beta-lactam pathway poses an interesting study in the evolution of pathways of secondary metabolites and the horizontal transfer of these pathways not only to other procaryotic organisms but to the lower fungi as well.

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2. Burnham, M. K. R., A. J. Earl, J. H. Bull, D. J. Smith, and G. Turner. 1988. European Patent Publication. 0 320 272.

3. Kovacevic, S., B. J. Weigel, M. B. Tobin, T. D. Ingolia, and J. R. Miller. 1989. *J. Bacteriol.* 171:754-760.

CC 015 STREPTOMYCIN BIOSYNTHESIS, Klaus Pissowotzki, Kambiz Mansouri-Taleghani and Wolfgang Piepersberg, Department of Microbiology, University of Wuppertal, Gauss-Str. 20, D-5600 Wuppertal, Fed. Rep. Germany

Streptomycin (SM) production genes are under investigation from Streptomyces griseus and S. glaucescens. Fourteen putative genes have been identified so far by DNA sequencing in the main cluster of genes from S. griseus. The direction of transcription varies among transcription units. The genes primary structures have several features in common such as similarities in ribosome binding sites, avoidance to use special codons such as TTA and CCA or the stop codons TAA and TAG, and strong base neighbourhood preferences. Protein comparisons on the primary structures of postulated str gene products revealed several indications on their possible functions. Regulation by antitermination of the str genes by the product of an internal regulatory gene, strR, will be discussed.
Two SM resistance genes, coding for SM phosphotransferases, were detected in S. griseus. One, aphD, clustered with the known production genes, and a second non-clustered resistance gene, aphE, share common primary structure motifs with the product of strN, one of the presumed SM production genes and with eucaryotic protein kinases. The implications of this finding for the evolution of secondary metabolic genes in streptomycetes and for possible functions of antibiotic-like products will also be discussed.

CC 016 MAINTENANCE OF CLONED TYLOSIN BIOSYNTHETIC GENES IN STREPTOMYCES FRADIAE ON FREELY-REPLICATING AND INTEGRATIVE PLASMID VECTORS, K. L. Cox and E. T. Seno, Department of Molecular Genetics, Lilly Research Laboratories, Indianapolis, IN 46285.

The biosynthesis of tylosin by Streptomyces fradiae is limited by the rate of methylation of the immediate precursor, macrocin, by the enzyme macrocin O-methyltransferase (MOMT). In an effort to relieve this rate limitation, the gene (tylF) coding for MOMT was cloned and, together with several other tylosin biosynthetic (tyl) genes, was introduced into a tylosin production strain on the multicopy plasmid vector, pIJ702. The resulting recombinant strain had several-fold enhanced levels of MOMT, produced more tylosin, but less total macrolide (tylosin + precursors) than control. Further investigation revealed that transformants with pIJ702 and some other plasmid vectors also produced less macrolide antibiotic than the untransformed parent strain. In an attempt to circumvent this problem, a number of approaches for integrating cloned genes stably into the genome were investigated. Two plasmids containing cloned S. fradiae DNA sequences on an unstable plasmid vector were integrated by homologous recombination into the genomic tyl cluster or near the genomic tlrA gene. Integration events at the latter site were considerably more stable than those in the tyl cluster. Vectors based upon the Streptomyces coelicolor minicircle (pIJ4210) and the integrative Streptomyces ambofaciens plasmid, pSAM2 (pKC684), transformed and integrated site-specifically into the genomes of restriction-deficient S. fradiae mutants, but they could not be introduced into production strains. A vector, pKC796 (N. Rao, S. Kuhstoss and M. Ballou, unpublished data), containing a fragment of ϕC31 DNA transformed and integrated stably at a unique site in tylosin-producing strains of S. fradiae. A number of recombinant plasmids containing cloned tyl genes in pKC796 were evaluated for effects on tylosin biosynthesis in S. fradiae. Although derivatives of pKC796 containing the cloned tylF gene caused greater MOMT activity and higher tylosin production than the parent strain, total macrolide production was lower, and this suppressive effect was associated with the pKC796 vector.

CC 017 REGULATION OF ANTIBIOTIC BIOSYNTHESIS IN STREPTOMYCES HYGROSCOPICUS.

Tom Holt, Anna Raibaud, Christine Laurent-Winter, Cecile Chang*, Phyllis Meyers*, Takeshi Murakami**, Julian Davies, and Charles Thompson, Institut Pasteur, Paris, *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, **Meiji Seika Kaisha, Tokyo

We are studying the biosynthesis of the antibiotic bialaphos in Streptomyces hygroscopicus as a model system to investigate genetic and regulatory aspects of secondary metabolism. The steps in the biosynthetic pathway of bialaphos and the corresponding biosynthetic genes (bap) have been well-defined. We are now focusing on the more fundamental aspects of the developmental process leading to antibiotic production during stationary growth phase. Expression of these genes is dependent on a regulatory gene (brpA) and shows a temporal correlation with the stringent response.

Computer-assisted analysis of high resolution two dimensional (2D) gel electrophoresis is being used to provide a global view of gene expression throughout the growth cycle. Over 20 proteins have been identified as bap gene products since they are not expressed in a regulatory mutant (brpA$^-$). Using cultures pulse-labeled with ^{35}S-methionine at two-hour intervals and analyzed on 2D gels, we have examined the overall pattern of gene expression in cultures from early log-phase growth to late stationary-phase growth. Semi-quantitative analyses of time-course experiments indicate that the bap proteins fall into at least five coordinately regulated families (Classes I-V). A transition occurs in the expression of Class I-IV proteins concurrent with a dramatic transient increase in ppGpp concentration. This preceeds the appearance of antibiotic in the medium by about four hours. The fact that the ppGpp response was also found in the brpA$^-$ mutant indicates that brpA does not control the stringent response however, the stringent response may lead to the activation of brpA.

In order to address the question of how brpA is controlled and how it activates bap genes, we have sequenced the brpA gene and localized its transcriptional start sites. Analysis of the deduced amino acid sequence of BRPA using several models for predicting hydropathicity suggests that it has three transmembrane domains at the N-terminus and a DNA binding consensus sequence at the C-terminus. This implies that BRPA may function as a membrane-bound signal transducer. It is of fundamental importance for us to understand what these signals are and how they allow BRPA to activate bialaphos biosynthesis.

Antibiotic Resistance and Biosynthesis

CC 018 INTERPLAY OF NOVOBIOCIN-SENSITIVE AND -RESISTANT DNA GYRASES IN SELF PROTECTION OF THE NOVOBIOCIN PRODUCER, STREPTOMYCES SPHAEROIDES. Eric Cundliffe, Department of Biochemistry and Leicester Biocentre, University of Leicester, Leicester, LE1 7RH, UK. Novobiocin, a coumarin antibiotic, inhibits bacterial DNA gyrase by binding to the B subunit of the A_2B_2 tetrameric enzyme. Gyrase is a DNA topoisomerase that uses the energy of ATP hydrolysis to introduce negative supercoils into closed circular DNA in vitro, although the role of the enzyme within the cell appears to be somewhat more complicated. The novobiocin-producing organism, Streptomyces sphaeroides, possesses two copies of gyrB, the gene encoding DNA gyrase B protein, whose products differ in their response to the drug. Novobiocin-sensitive DNA gyrase is produced constitutively in this strain whereas resistant gyrase appears when growth takes place in the presence of the drug. The promoter isolated from the novobiocin-resistance determinant gyrB^R responds sharply to changes in DNA topology, being activated when the superhelical density is reduced and vice versa when supercoiling of DNA is increased. It is proposed that resistance to novobiocin in S. sphaeroides is induced by a reduction in DNA supercoiling due to the action of autogenous drug on the sensitive gyrase.

CC 019 ACQUIRED PENTALENOLACTONE RESISTANCE OF STREPTOMYCES ARENAE BY DIFFERENTIAL EXPRESSION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ISOZYMES, Kai-Uwe Fröhlich, Michael Wiedmann and Dieter Mecke, Physiologisch-chemisches Institut, Universität Tübingen, Hoppe-Seyler-Str. 1, 7400 Tübingen, Federal Republic of Germany

The antibiotic pentalenolactone (PL) selectively and irreversibly inactivates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of all organisms tested. The producer strain Streptomyces arenae expresses a resistant GAPDH during the production phase, otherwise, a PL-sensitive GAPDH is formed. While PL-insensitive GAPDH is of similar molecular weight as GAPDH of other organisms (37,000 per subunit), PL-sensitive GAPDH from S.arenae has an unusual size (43,000 per subunit). Differences in the aminoterminal sequence and the low immunological crossreactivity indicate the existence of two GAPDH genes. Both genes were cloned in Escherichia coli and identified by Western hybridisation and in vitro gene expression. In clones of the PL-sensitive gene, tetramers of the E.coli and S.arenae enzyme coexisted independently as shown with activity staining following nondenaturating gel electrophoresis. Clones of the PL-insensitive GAPDH gene contained hybrid tetramers of both E.coli and S.arenae GAPDH subunits. The hybrid enzyme exhibited an intermediate PL-sensitivity compared to E.coli and PL-insensitive S.arenae GAPDH and could be purified to homogeneity.

Morphological Differentiation

CC 020 MOLECULAR GENETIC ASPECTS OF THE FORMATION OF SPORE CHAINS IN AERIAL HYPHAE, Keith F Chater, Nigel K Davis, Tan Huarong, Kitty A Plaskitt, Juan Soliveri and Celia J Bruton. John Innes Institute, Colney Lane, Norwich NR4 7UH, UK

Streptomyces coelicolor colonies produce a white aerial mycelium after a few days on agar media. The aerial mycelium turns grey as individual aerial hyphae metamorphose into chains of spores. Mutants that remain white on prolonged incubation have been used to identify eight whi loci that are essential for normal sporulation. Three whi loci have been cloned and analysed. The whiG locus encodes a sigma factor, whose overproduction switches hyphae from growth to sporulation[1]. Some progress has been made in isolating promoters that are whiG dependent. The whiB locus, needed for sporulation septation, encodes a small protein rich in charged residues and cysteines. The whiE locus, which is needed for spore pigmentation, is a cluster of genes for biosynthesis of an unidentified polyketide compound. Evidence that some of these genes influence glycogen metabolism during spore formation will be discussed.

[1]Chater, K.F. et al. Cell, October 1989, in press.

CC 021 PROBING Streptomyces MORPHOLOGICAL DEVELOPMENT WITH Tn4556 AND LUCIFERASE, Alan Schauer, Hana Im, Charles Sohaskey, Andrea Nelson, James Daniel and Gregg Helt, Department of Microbiology, University of Texas at Austin, ESB 226, Austin, TX 78712. We have begun a genetic analysis of S. colelicolor morphological development and transcriptional regulation. A model late gene, sapA, is responsible for synthesis of a spore-coat protein. As spores are not synthesized until relatively late in the growth cycle, the sapA promoter is not switched on until spores are needed. The promoter sequence is most unusual and is apparently recognized by a specialized form of RNA polymerase. The sapA promoter is being mutagenized to identify base-pairs and structures that are critical for its strength and regulation. Second-site mutations which alter sapA transcription have been isolated. Normally, P_{sapA} is induced very late in development, after aerial hyphae are visible. In the two independent mutants isolated thus far, light production is strongly induced during vegetative colony growth and no further induction has been detected at the normal time. By curing the mutants of plasmid and then reintroducing the plasmid-based P_{sapA}-lux fusion, we have shown that the mutations are located on the host chromosome. Additional experiments ruled out some mechanisms for premature light induction in the mutants. We are currently attempting to clone the responsible gene(s) by complementation.

The second center of activity is the construction and application of a gene-fusion transposon for Streptomyces. Tn4556 was originally isolated from Streptomyces fradiae and is a member of the Tn3 family of elements which transpose via a replicative, cointegrate mechanism. Derivatives of this transposable element can simultaneously inactivate target genes and place the light-encoding luciferase genes under control of the exogenous transcription unit. In this way, we can generate, clone and analyze developmental mutants very rapidly. We have isolated a number of morphological mutants of Streptomyces coelicolor with Tn4556; these are now being compared and mapped against the known sporulation genes.

Transcriptional Control

CC 022 HERBICIDE INDUCIBLE GENE EXPRESSION IN STREPTOMYCES GRISEOLUS ATCC11796, Charles A. Omer, Nina V. Patel, Ken Leto, James Romesser, Patricia Harder and Daniel P. O'Keefe. Central Research and Development Department, E. I. DuPont, Experimental Station, Wilmington, DE 19800-0173. *Streptomyces griseolus* ATCC11796 is capable of metabolizing a class of herbicidal chemicals known as sulfonylureas. It does this via two cytochromes P450 named $P450_{SU1}$ and $P450_{SU2}$ which are the products of the *suaC* and *subC* genes respectively. We have cloned and sequenced the *suaC* and *subC* genes along with the genes(*suaB* and *subB*) encoding the ferredoxins that transfer electrons to these cytochromes P450. Each cytochrome P450 gene is expressed in an operon along with the gene for its corresponding ferredoxin. The two operons are quite similar to each other at the amino acid and DNA sequence levels although the two operons are not near one another on the *S. griseolus* genome. Expression of these genes in *S. griseolus* is transcriptionally regulated with sulfonylureas acting as inducers. Regulation of expression of the *suaC, B* and *subC, B* transcripts appears to be repressor controlled, but the *suaC, B* and *subC, B* transcripts are regulated independently of one another. We have identified a DNA binding protein that binds to two inverted repeats upstream of the *suaC* coding sequence. This DNA binding protein appears to be induced in the presence of sulfonylureas. We are investigating how this possible modulation of a DNA binding protein could be involved in regulation of the *suaC* and *B* genes. We have identified a separate DNA binding protein that binds upstream of the *subC* coding sequence. Expression of this DNA binding protein is constitutive and it appears to behave as a typical repressor.

CC 023 EXPRESSION AND SECRETION OF AN EXTRACELLULAR ESTERASE FROM *STREPTOMYCES SCABIES*, Janet L. Schottel, Greg Raymer, Michael McGrew, and Leslie Harmon, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108

Streptomyces scabies is the causative organism for scab disease on a variety of underground vegetables (1). An extracellular esterase produced by the pathogen has been identified (2) which may be involved in degrading suberin, the waxy polyester compound covering underground plant parts (3). The esterase gene has been cloned and sequenced. The levels of esterase mRNA produced by the pathogen is influenced by zinc which is a natural component of the suberin. The untranslated sequence 5' to the structural gene has been subcloned into the pIJ486 plasmid (4). Using the neomycin resistance gene as a reporter, deletions have been introduced into this sequence to begin to localize the RNA polymerase binding site and the putative zinc regulatory sequences.

The *S. scabies* esterase gene has been cloned and expressed in *S. lividans*, and the esterase is secreted in this organism. The esterase gene has also been expressed in *E. coli*. Esterase activity was detected in the periplasmic space indicating that this protein is secreted in *E. coli*. Native polyacrylamide gel analysis of periplasmic proteins and *in situ* esterase activity assays have revealed three esterase species secreted into the periplasm. One of these esterases appears to be very similar to the secreted esterase in *S. scabies*, suggesting a site for signal peptide cleavage that is commonly recognized by both organisms. The additional esterases may represent the recognition of alternate processing sites in *E. coli* or the secretion of an unprocessed esterase.

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CC 024 MULTIPLE GENES FOR PRINCIPAL SIGMA FACTOR AND MORPHOLOGICAL CHANGES OF Streptomyces coelicolor A3(2), Hideo Takahashi, Kan Tanaka and Tetsuo Shiina, Institute of Applied Microbiology, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan. Mycelial Streptomyces strains undergo complex morphological and biochemical changes during the life cycle. To understand the control mechanisms of gene expression in the mycelial and biochemical differentiation of Streptomyces cells, it is essential to clarify the transcriptional machinery. In the normal cell state of Escherichia coli and Bacillus subtilis, RNA polymerase holoenzymes contain a principal sigma factor (rpoD gene product) that plays a central role in the transcriptional initiation of 'house keeping' genes. We have previously shown that a 29-base oligonucleotide probe designed from a conserved amino acid stretch of the known principal sigma factors was successfully used to identify the rpoD gene homologs of eubacteria. We have cloned and sequenced the DNA fragments corresponding to the four signals from S. coelicolor A3(2). Open reading frames found in the DNA regions were named hrd(homologs of rpoD gene), which had extensive homology with rpoD gene products of E. coli and B. subtilis. Computer analyses of the hrd nucleotide sequences and the deduced amino acid sequences indicated that the homology among the four hrd genes and gene products with those of E. coli and B. subtilis was extensive throughout the entire region of the genes. It was strongly suggested that they have evolved from the same ancestor. The hrd gene products of S. coelicolor A3(2) were shown to have two probable helix-turn-helix domains (HTH motifs) at the exactly same interval from the 'rpoD box' in the B subdomian. Northern hybridization analyses of the mRNA from the four hrd genes have indicated that all of the four genes are expressed differently corresponding to the mycelial and biochemical differentiation of S. coelicolor cells.

1.Tanaka,K.,T.Shiina and Takahashi,H. Science 242,1040(1988)
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CC 025 TRANSCRIPTIONAL REGULATION OF THE *STREPTOMYCES* GALACTOSE OPERON. J. Westpheling[1], M. Brawner[2], Jim Fornwald[2], Dan-Yang Huang[1], Cheryl Ingram[1], Sibylle Mattern[1], University of Pennsylvania Medical School, Philadelphia[1], SmithKline and French Laboratories, King of Prussia[2]

A promoter, *galP1*, located at the 5' end of the *Streptomyces gal* operon, is responsible for glucose-sensitive, galactose-dependent transcription of the galactose utilization structural genes. Attempts to dissect the transcriptional machinery involved in the regulation of *galP1 in vitro* have identified a new form of RNA polymerase holoenzyme with a sigma subunit of approximately 30,000 daltons (apparent molecular weight as determined by migration in polyacrylamide gels).This polymerase may be one of four recently described enzymes in *Streptomyces* that may recognize promoters with nearly homologous recognition sequences.

Using oligonucleotide-directed mutagenesis, we have identified mutations within *galP1* that affect promoter function and regulation *in vivo*. The effects of these mutations were analyzed by making transcriptional fusions to the *Pseudomonas putida xylE* gene and measuring the activity of catechol dioxygenase. There are two striking features of the DNA sequence of *galP1* we have shown to be important to promoter function and regulation: six consecutive guanines centered around -35 and a series of hexamers that conform to the consensus TGTGAT. Single base changes within the guanines either greatly increase expression from *galP1* or eliminate it entirely. Changes within the TGTGAT hexamers result in expression from *galP1* in the absence of inducer. We suggest that these TGTGAT hexamers act as an operator for *galP1* and that they bind repressor in a coordinate fashion. *In vitro* analysis of mutations in the consecutive guanidyl residues at -35 indicate an unusual interaction between RNA polymerase and the DNA sequence upstream of the *galP1* transcription start site.

In addition we have isolated mutations that identify genes whose products act in *trans* to regulate transcription from *galP1*. We are in the process of cloning and characterizing these genes.

Banquet Address

CC 026 TRANSLATIONAL INITIATION, Larry Gold, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347

All eubacteria may initiate translation and regulate gene expression post-transcriptionally using mechanisms also found in E. coli. Conversely, E. coli might represent an organism that is both well-studied and irrelevant, at least to those concerned with similar questions in the streptomycetes. I will outline my view of each and every aspect of E. coli translation, its regulation, and other post-transcriptional regulatory phenomena and contrast those systems with similar systems in the streptomycetes.

Gene Transfer

CC 027 TRANSDUCTION AND TRANSPOSITION IN STREPTOMYCES, Richard H. Baltz, Margaret A. McHenney, Donald R. Hahn and Patricia J. Solenberg, Department of Molecular Genetics, Lilly Research Laboratories, Indianapolis, IN 46285

Plasmid pRHB101 contains a segment of bacteriophage FP43 DNA inserted in the SphI site of pIJ702 which causes it to be transduced efficiently by FP43. Insertion of a 1.7 kb segment of Saccharopolyspora erythraea DNA containing an erythromycin resistance gene (ermE) into the KpnI site of pRHB101 had little effect on transduction frequencies, and ermE was expressed in several streptomycete hosts. pRHB101 has been modified to contain a multiple cloning site inserted in the KpnI site to facilitate the construction of transducible plasmids containing inserts of antibiotic biosynthetic and other genes. Restriction mapping studies indicate that the 8 kb segment of FP43 DNA that facilitates transduction contains a pac site. We also constructed transposable elements that contain an apramycin resistance gene from Escherichia coli inserted between two open reading frames in IS493, a new IS element from S. lividans. The resulting elements, Tn5096 and Tn5097, transposed from replicating and non-replicating plasmids into several sites in S. griseofuscus.

CC 028 MOLECULAR BIOLOGY OF Tn4556 AND ITS APPLICATIONS, Shiau-Ta Chung and Lori L. Crose, Bioprocess Research and Development, The Upjohn Company, Kalamazoo, MI 49001

The transposon Tn4556 was discovered in a neomycin-producing strain of Streptomyces fradiae (1). Tn4556 has been shown to contain terminal inverted repeat sequences (IRs) of 38 base pairs (bp) with a single mismatched pair. The Tn4556 IRs are 70% homologous with the ends of Tn3. Tn4556 generates 5-bp direct repeat sequences during transposition (2). This transposon is 6,625 bp in length and contains nine possible open reading frames. After a viomycin resistance gene (vph) was cloned into Tn4556, the Tn4556::vph (Tn4560) was demonstrated to transpose randomly from plasmid to plasmid, plasmid to chromosome, and vice versa. Tn4560 was also demonstrated to mutagenize genomic DNA and DNA fragments of Streptomyces lincolnensis cloned either on plasmid pIJ916 or pIJ912 in Streptomyces lividans. The mutated DNA on the plasmid was reintroduced into S. lincolnensis and induced to replace the wild-type genomic DNA. Using this site-directed Tn4556 mediated transplacement mutagenesis, we are able to identify and analyze lincomycin biosynthetic genes.

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(2) Olson, E.R., and S.T. Chung. 1988. Tn4556 of Streptomyces fradiae: nucleotide sequence of the ends and the target sites. J. Bacteriol. 170: 1955-1957.

CC 029 INTERGENERIC DNA TRANSFER AND ITS EVOLUTIONARY CONSEQUENCES,
Julian Davies, Institut Pasteur, Department of Biotechnology, Paris

In this presentation, I would like to ask the following questions :

1) What is the evidence for inter-specific DNA transfer in Nature ?
2) In what way is functional gene transfer related to DNA transfer ?
3) What mechanisms promote natural DNA transfer ?
4) What environmental factors influence natural DNA transfer ?
5) What are the potential evolutionary consequences of DNA transfer ?

Studies of antibiotic resistance genes and antibiotic biosynthetic pathways in different organisms may provide partial answers to some of these questions and will be discussed.

CC 030 PHYSICAL CHARACTERIZATION OF SCP1 AND ANALYSIS OF ITS INTEGRATION MECHANISM INTO THE CHROMOSOME OF S. COELICOLOR,
Haruyasu Kinashi, Miyuki Shimaji and Tadashi Hanafusa, Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan

PFGE analysis enabled the detection of giant linear plasmids from several antibiotic-producing strains of Streptomyces species (1). SCP1 was shown to be a giant linear plasmid of 350 kb (2) and to form a series of plasmids by integration of SCP2 molecules in S. coelicolor JCM4979 (3). Our recent studies have revealed physical characters of SCP1 and its integration mechanism into the chromosome, which are presented here.

Physical map of SCP1 was constructed by extensive digestion analysis and cloning, which indicated the presence of unusually long terminal inverted repeats of about 70 kb long on both ends. Terminal inverted repeats so far found in small linear plasmids are shorter than 1 kb. Exonulease digestion suggested that the 5'-ends of SCP1 might be blocked by a protein as in the cases of other linear plasmids. The terminal fragments of SCP1 were cloned and their nucleotide sequences were determined. Six clones analyzed had an identical sequence except for the length of the terminal Cs: ...CCGTTAGGCCTCTCCGCCCC(CC)-3'. The difference of the length of the terminal C chain might represent the real heterogeneity of the ends or might have been formed by a nuclease attack during the cloning process. In this terminal region, 82% (9/11) base identity was observed between SCP1 and pSLA2, a linear plasmid isolated from S. rochei (4).

SCP1 is also known to be present in an integrated form in NF strains of S. coelicolor. To know the integrated structure of SCP1, we tried to clone the junctions between SCP1 and the chromosomal DNA in an NF strain, S. coelicolor 2612. A clone containing one of the junctions was selected by hybridization to a terminal fragment of SCP1 used as a probe. Sequencing of this clone indicated that an almost intact terminal structure of SCP1 was preserved in this junction. Cloning and sequencing of the other junction revealed occurrence of a deletion covering a terminal region of SCP1 and a fragment of the chromosomal DNA. From these data, a model of the SCP1 integration was deduced, which will be discussed.

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CC 031 THE INTEGRATIVE CONJUGATIVE ELEMENT pSAM2 OF *Streptomyces ambofaciens*.

J-L. Pernodet, T. Smokvina, F. Boccard and M. Guérineau. URA CNRS D1354. Université Paris-Sud, F-91405 Orsay Cedex, France.

pSAM2 is a 11 kb element present in an integrated state in *S. ambofaciens* ATCC23877 and both integrated and as a plasmid in a mutant of this strain (1). The strain *S. ambofaciens* DSM40697 is devoid of pSAM2. pSAM2 is self-transmissible and mobilizes chromosomal markers (2). After transformation or transfer following interspecific crossing, pSAM2 is able to integrate at a specific site in the genome of different *Streptomyces* species (3,4). The integration of pSAM2 occurs by site-specific recombination between the plasmid attachement site (*att*P) and the (*att*B) site of the host genome. As a result of integration, two attachement sites, *att*L and *att*R are generated on the left and right sides, respectively, of the pSAM2 sequence. All *att* sites share a 58-bp identical core sequence (5). The site-specific recombination system of pSAM2 presents strong similarities with the one of several temperate bacteriophages (6). The integration event is promoted by a site-specific recombinase of the integrase family. The *int* gene encoding this integrase is close to *att*P. A small open reading frame is located upstream of the *int* gene, and the predicted protein exhibits similarities with excisionases involved in the excision of temperate phages (7) or of conjugative transposons (8). In *S. ambofaciens* ATCC23877, the integrated pSAM2 is flanked by *att*L and *att*R, but a third *att* sequence (*att*X) is present in this strain; and *att*X and *att*L are the boundaries of a 43 kb region which is absent in the *S. ambofaciens* strain devoid of pSAM2. Integrative cloning vectors derived of pSAM2 have a large host range (5,9) and sequences hybridizing to the *att* site of pSAM2 seem to be widespread among *Streptomyces* (5) and even among actinomycetes. The structure of the *att* site will be discussed.

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Genome Structure, Plasticity and Modifications

CC 032 MOLECULAR GENETIC ANALYSIS OF THE PHASE VARIATION OF THE PHAGE GROWTH LIMITATION (pgl) LOCUS OF STREPTOMYCES COELICOLOR A3(2) AND THE MECHANISM OF PHAGE RESISTANCE, Carole Laity, Keith F. Chater and Mark J. Buttner, John Innes Institute, NORWICH NR4 7UH, UK.

Wild-type S.coelicolor is resistant to plaque formation by the Streptomyces phage øC31 (the Pgl^+ phenotype). However, Pgl^+ strains give rise to Pgl^- derivatives (which permit plaque formation by øC31) at a frequency of 10^{-2} to 10^{-3} per spore and Pgl^- strains revert at a similar frequency[1]. This phase variation has only been identified in S.coelicolor, for instance all strains of S.lividans are sensitive to øC31. In addition, the Pgl system is highly specific for øC31, in that no other Streptomyces phages have been identified that plaque on Pgl^-, but not on Pgl^+ strains of S.coelicolor. The Pgl phenotype is of interest not only because of its phase variation but also because of the mechanism by which it prevents phage propagation. øC31 phages grown on a Pgl^- strain of S.coelicolor or on a different streptomycete can lyse (or lysogenise) an individual hyphal compartment of a pgl^+ strain but the phages released from this first round of infection are modified in such a way that they are unable to infect neighbouring compartments, hence the absence of plaque formation[1]. These modified phage are, however, capable of infecting a Pgl^- strain[1]. This system therefore represents a reversal of a classical restriction-modification pattern.

We have cloned a component of the Pgl system which includes the genetically unstable locus. The clone confers resistance on Pgl^- strains of S.coelicolor but it does not confer resistance on the naturally phage-sensitive strain S.lividans. The pgl^- allele of the pgl locus has also been cloned and has an identical restriction map to the pgl^+ allele. It therefore seems unlikely that a DNA rearrangement event such as a DNA segment inversion is responsible for the observed phase variation.

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CC 033 GENETIC INSTABILITY IN STREPTOMYCES LIVIDANS 66

John Cullum, Peter Kaiser*, Fiona Flett*, Matthias Redenbach and Uwe Rauland, LB Genetik der Universität Kaiserslautern, Paul-Ehrlich-Str. 22, D-6750 Kaiserslautern, Federal Republic of Germany and Department of Biochemistry, U.M.I.S.T., Manchester, United Kingdom.

The chloramphenicol resistance gene of S. lividans 66 is unstable and gives rise to sensitive mutants (Cml^S) (which have deleted 250-300 kb of chromosomal DNA) at a frequency of about 1% per spore. The Cml^S mutants are very unstable, producing arginine auxotrophs (Arg^-) at a frequency of 25% per spore. The Arg^- strains have deleted 250-300kb of additional chromosomal DNA and have also amplified a 5.7kb chromosomal DNA sequence to several hundred tandem copies. The mechanism of this genetic instability has been investigated by introducing selectable marker genes into the regions subject to deletion and determining whether deletion or amplification of the marker gene is essential for the observed instability.

A new seemingly independent instability phenomenon has been discovered in S. lividans 66. Strains that have been selected for transposition of the element IS466 often show chromosomal DNA amplification (about 30 % of strains). The amplified regions are different in different strains, but sometimes overlapping.

CC 034 GENOME REARRANGEMENTS IN *STREPTOMYCES GLAUCESCENS*,

Alex Häusler, Ashley Birch and Ralf Hütter, Institute of Microbiology, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland

Streptomycetes exhibit an unusually high degree of genetic instability, a phenomenon which is marked by the high frequency loss of certain phenotypic traits and which involves gross genomic rearrangements. Deletion of structural genes has been found to be responsible for the loss of such traits which include biosynthesis of and resistance to antibiotics, sporulation factors, pigment synthesis and arginine biosynthesis (1). In *S.glaucescens* the phenotypically easily recognizable melanin formation and resistance to streptomycin are prone to genetic instability, being lost at high frequency as a result of extensive genomic deletions. In addition, mutant strains possessing deletions of both structural genes (*melC* and *strS*) frequently display intense DNA amplifications. These amplified DNAs (ADSs) were heterogeneous for location, copy number and the sequences involved and originated from a single 100 kb region of the chromosome called the "AUD locus" (2). With the help of a wild type cosmid library it was possible to link the two unstable loci and the amplified DNAs within a 600 kb segment of the chromosome and in addition, it could be shown that the previously characterized deletions were components of much larger deletions ranging from 270 kb up to 900 kb which were responsible for the sequential loss of the *strS* and *melC* loci (3). The longest deletions analysed comprised approximately 15-20% of the chromosome and are to our knowledge the largest prokaryotic deletions characterized to date. Amplified DNA was only present in those strains containing the most extensive deletions which terminated either close to or directly adjacent to the amplifiable units of DNA (AUDs). Analysis of amplification and deletion formation on the sequence level revealed the presence of only short, imperfect "microhomologies" at various AUD termini and deletion endpoints respectively. These results indicate that an illegitimate recombination mechanism may have been involved in the formation of these events and that the observed heterogeneity can be attributed to the absence of long sequence repetitions. In some of the cases investigated, short homologies were found between deletion termini of different mutant strains indicating that specific DNA sequences may play a role in deletion formation.

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(2) Häusler *et al.*, (1989) Mol. Gen. Genet. **217:** 437-446.
(3) Birch *et al.*, (1989) Mol. Gen. Genet. **217:** 447-458.

CC 035 THE Streptomyces coelicolor GENOME, David A. Hopwood, Helen M. Kieser and Tobias Kieser, John Innes Institute, Norwich NR4 7UH, U.K.
The genome of S. coelicolor shows several interesting features. These include two long "silent" regions of the linkage map devoid of genetic markers; a circular symmetry in the arrangement of genes of related function; and a series of integrating genetic elements (including the large linear SCP1 plasmid, the plasmidogenic SLP1 sequence, the øC31 prophage, IS110, IS466 and the mini-circle). In common with those of many other Streptomyces spp., the S. coelicolor chromosome also has segments of amplifiable and deletable DNA. To analyse such genomic features, as well as to provide a resource for those working with this genetically most studied streptomycete, we are constructing a physical map of the chromosome by pulse field gel electrophoresis and relating it to the genetic map. Progress on the project will be reviewed.

Expression Systems

CC 036 EXPRESSION OF THE SOLUBLE CD-4 RECEPTOR IN STREPTOMYCES. Mary Brawner, Dean Taylor, and Jim Fornwald, Gene Expression Sciences, Smith Kline and French Laboratories, King of Prussia, PA 19406
The human T-cell receptor CD-4 acts as a recognition element in major histocompatibility complex class II responses and as a receptor for the HIV virus. A soluble derivative of CD-4, sCD-4, lacks the transmembrane and cytoplasmic domains but is still capable of binding HIV thereby preventing HIV infection and syncytium formation in vitro. These features make sCD-4 an attractive therapeutic to control the progression of AIDS. At present, production of active sCD-4 relies on expression by chinese hamster ovary cells. Expression of sCD-4 by a bacterial host is desirable since this should be a more efficient and cost effective mode of production. The choice of bacterial hosts, however, is limited to those capable of producing active, disulfide-bonded proteins since sCD-4 is a complex protein containing three intramolecular disulfide bonds. We have previously shown that S. lividans and S. longisporus naturally produce the protein protease inhibitors LEP-10 and LTI, respectively. Both LEP-10 and LTI are made at high levels, efficiently exported into the culture supernatant, and like sCD-4, are disulfide crosslinked proteins. The LTI promoter and its signal sequence have been used to direct expression and export of sCD-4 and its derivatives in S. lividans. Streptomyces produced sCD-4 and various truncates are biologically active as judged by immunopreciptation using various monoclonal antibodies which are sensitive to protein conformation and inhibit HIV binding to T-cells. Our current efforts are focused on increasing the expression levels of sCD-4. Some factors which affect expression levels and sCD-4 export will be discussed.

CC 037 XYLOSE ISOMERASE FROM *STREPTOMYCES RUBIGINOSUS*: CLONING, NUCLEOTIDE SEQUENCE, GENE REGULATION AND ENZYME ENGINEERING.

H.C. Wong, H.C. Lin, F.L. Reichert, and R.J. Drummond, Cetus Corporation, 1400 Fifty-Third St., Emeryville, CA 94608

A 4-kb fragment of the *Streptomyces rubiginosus* chromosome that contains the gene for xylose isomerase (*xylA*) was cloned into plasmid pBR322. The coding region of the gene on the recombinant plasmid was localized by Southern analysis using *xylA*-specific oligonucleotide probes, and the nucleotide sequence of a 2000-bp segment of DNA that contains the coding and the flanking regions of the gene was determined. A sequence of 388 amino acids, comprising a Mr 43,232 protein, was deduced from the single large open reading frame. Comparison the deduced amino acid sequence encoded by the open reading frame with the N-terminal amino acid sequence of xylose isomerase protein purified from *S. rubiginosus* confirmed that the cloned gene encodes xylose isomerase. When expressed under control of the trp promoter and trpLE translation initiation signal, the cloned gene is capable of complementing an *E. coli* xylose isomerase deficient strain.

The transcription regulation of the xylose isomerase gene in *S. rubiginosus* was investigated using a unique promoter probe plasmid that integrates into the genome. Our results indicate that the xylose isomerase gene is regulated negatively by glucose and positively by D-xylose. An open reading frame, transcribed from the opposite strand of DNA, has been found 114 bp upstream of the xylose isomerase gene. This open reading frame is preceded by a potential ribosome binding site. A promoter positioned at the 5' region of this open reading frame was identified. This promoter was also found to be regulated positively by D-xylose and negatively by glucose. Genetic disruption of this open reading frame is being carried out to clarify the role of this protein in D-xylose degradation in *S. rubiginosus*.

Xylose isomerase is a commercially important enzyme used in the production of glucose/fructose syrups from starch. The deduced amino acid sequence of *S. rubiginosus* xylose isomerase is highly homologous to that of xylose isomerases from several other microorganisms. Our deduced sequence has been used to interpret data from a high resolution X-ray crystal structure of the enzyme. The 1.6 Å crystal structure has been used to guide site-directed mutagenesis of the wild-type enzyme for the purpose of increasing thermostability and improving k_{cat} and K_m for the conversion of glucose to fructose. Mutant enzymes displaying increased thermostability and a reduced K_m for glucose will be described.

Late Addition

CC 038 UNDERSTANDING AND USING THE TIPA THIOSTREPTON-INDUCIBLE PROMOTER, José Caso*, Tom G. Holt, Takeshi Murakami**, Eriko Takano** and Charles Thompson, Institut Pasteur, Paris, France, *present address Universidad de Oviedo, Oviedo, Spain, **Meiji Seika Kaisha, Tokyo,

Thiostrepton induces expression of a family of at least four related proteins (apparent molecular weights: 17, 19, 30 and 56 kdal) in Streptomyces lividans. We have previously reported the cloning, sequencing, and preliminary studies of the regulation of the 19 kdal protein (TIPA) (1). Here we describe our more recent efforts to understand and use the tipA promoter. This promoter maps 347 bp upstream of the apparent TIPA translational initiation site. When a 143 bp fragment containing this region was cloned into the promoter-probe vector pIJ486, bidirectional thiostrepton-inducible promoter activity could be demonstrated in vivo. In vitro transcriptional run-off and gel retardation experiments using this fragment demonstr-ated an activatory factor which was only found in induced cultures. Purification of this activity identified it as the 30-kdal thiostrepton-induced protein. N-terminal analysis showed that the 30-kdal activator protein was encoded by a nucleotide sequence which began 19 bp downstream of the tipA transcriptional initiation site using an open reading frame which included the sequence encoding TIPA. Thus the 19-kdal TIPA protein is a truncated form of the 30-kdal regulatory protein (TIPA'). A computer search revealed that TIPA' belonged to a family of similar proteins which are Hg^{++} inducible and regulate mercury resistance in Neurospora, Pseudomonas, Serratia, and S. lividans (J. Altenbuchner, personal communication). Since TIPA is not identical to the merR gene product of S. lividans and the tipA promoter is not induced by mercury, we do not think that tipA' regulates mercury resistance in S. lividans but it does have a common evolutionary origin with the merR genes. The tipA promoter has also been used successfully to coordinately over-express six gene products encoded by a polycistronic transcript of the bialaphos antibiotic biosynthetic gene cluster. "Second generation" expression vectors are now under development.

1. Murakami, T., T. G. Holt, and C. J. Thompson. 1989. Thiostrepton-induced gene expression in Streptomyces lividans. J. Bacteriol. 171:1459-1466.

Primary and Secondary Metabolism; Transcriptional Control; Differentiation

CC 100 GENES AND CONTROL OF ARGININE METABOLISM IN STREPTOMYCETES, Simon Baumberg, Gabriel Padilla and Zoe Hindle, Department of Genetics, University of Leeds, Leeds LS2 9JT, U.K. We are studying the genetics and physiology of arginine biosynthesis and catabolism in streptomycetes, to acquire insight into controls of amino acid metabolism in this group and to determine whether such controls differ according to whether arginine is or is not a secondary metabolite precursor. Specific activities of ornithine carbamoyltransferase (OCT), arginase (AG) and guanidinobutyrate ureohydrolase (GBUH) were determined for wild-type S. coelicolor, S. lividans, S. griseus and S. clavuligerus grown with and without arginine, and for arginine auxotrophs of the first two grown under conditions of arginine limitation. In all cases, OCT levels were low and showed little (c. 2-3 fold) derepression; AG levels were low and showed no induction; and GBUH was arginine-inducible with high induced levels. Fragments of S. coelicolor DNA complementing uncharacterised Streptomyces arginine auxotrophs were subcloned in E. coli and complemented argB, C, E and H mutations. The S. coelicolor gene order appears to be C-E/J-B. Part of argC has been sequenced and shows limited homology with the E. coli and Bacillus subtilis sequences. Expression of these genes in E. coli may be from one or more promoters within the S. coelicolor DNA.

CC 101 CLONING AND CHARACTERISATION OF A LYSOZYME GENE FROM *STREPTOMYCES COELICOLOR* "MÜLLER" DSM3030, Elli Birr[1], Wolfgang Wohlleben[1], Alfred Pühler[1], Barbara Bräu[2], Rüdiger Marquardt[2], [1]Faculty of Biology, Universität Bielefeld, D-4800 Bielefeld, F.R.G. [2]Hoechst AG, D-6230 Frankfurt 80, F.R.G.

Streptomyces coelicolor "Müller" is known to excrete the lysozyme N-acetylmuramidase. Culture filtrates of this strain form a characteristic halo on agar plates containing freeze-dried *Micrococcus luteus* cells (lysoplate technique). The halo consists of a clear inner zone and a turbid outer ring. Simulation experiments suggested that the turbid outer ring is produced by lysozyme whereas the clear inner zone is caused by an additional protease action. Using the lysoplate technique UV- and NTG-mutagenized strains of *S. coelicolor* "Müller" were screened for mutants defective in lysozyme production. Two mutants, SC11 and SC12, were identified. The mutant SC11 was selected for complementation studies. Genomic DNA of *S. coelicolor* "Müller" digested with *Sau*3A and inserted into pEB15 was introduced into mutant SC11. A 2.9kb DNA fragment was identified which restored the lysozyme production of mutant SC11. According to the diameter of the halos the complemented mutant SC11 was suggested to produce more lysozyme than the wildtype strain. (E. Birr et al., Appl Microbiol Biotechnol (1989) 30:358-363).

CC 102 CENTRAL METABOLIC PATHWAYS IN STREPTOMYCES COELICOLOR AND THE PRODUCTION OF ANTIBIOTIC PRECURSORS, Helena Bramwell, Hugh G. Nimmo, Iain S. Hunter and John R. Coggins, Departments of Biochemistry and Genetics, Glasgow University, Glasgow G12 8QQ. Scotland.

Despite detailed knowledge of both the biochemistry and molecular biology of antibiotic biosynthesis little is known about the primary metabolic pathways which provide precursors for these molecules. In an attempt to define the important primary biosynthetic pathways in S.coelicolor we have assayed cell free extracts of the organism, grown on defined medium with glucose as sole carbon source, for several anaplerotic enzymes. Of these only phosphoenolpyruvate carboxylase (PEP carboxylase) has been detected suggesting that it plays a major role in sustaining flux to biosynthesis in this organism. Purification and preliminary characterisation of PEP carboxylase from S.coelicolor show that it is of a similar size to other bacterial PEP carboxylases and shares some of their regulatory properties. The major antibiotics produced by S.coelicolor, actinorhodin and undecylprodigiosin are both of polyketide origin and are thus derived from malonyl CoA. We have investigated two alternative pathways by which malonyl CoA could be produced in S.coelicolor. The results of these investigations, the characteristics of S.coelicolor PEP carboxylase and the significance of each of the reactions to antibiotic biosynthesis are discussed.

CC 103 **CLONING AND CHARACTERIZATION OF THE *Sph* I RESTRICTION MODIFICATION SYSTEM FROM *Streptomyces phaeochromogenes***

J.E. Brooks, L. Sznyter, C. Vaccaro, M. Arnaud, T. Jager-Quinton, G. Wilson, L. Moran, B. Slatko, and V. Bernan, Research Division, New England Biolabs, Beverly, MA 01915.

Sph I, a Type II restriction modification from *Streptomyces phaeochromogenes*, recognizes the sequence GCATGC. The endonuclease cleaves at GCATG↓C to generate a 3' four base overhang. A 5.4 kb *Pst* I fragment from the *S. phaeochromogenes* genome has been cloned into pBR322 and shown to express the *Sph* I methylase at low level in *E. coli*. Clones carrying the fragment have no endonuclease activity. Extensive mapping and subcloning have been used to determine the position and orientation of the *m* gene. The nucleotide sequence of the region is now being determined. The entire 5.4 kb fragment and also various restriction fragments have been transferred into *S. lividans* on pIJ486, pIJ487 and also the low copy vector pIJ922. Their expression in *Streptomyces* will be discussed.

CC 104 MUTATIONAL RESISTANCE TO DNA INTERCALATING COMPOUNDS IN *STREPTOMYCES LIVIDANS*: MAPPING OF THE MUTATIONS AND CLONING OF THE MUTANT GENES, Carton W. Chen[1,2], Yi-Jiun Huang[1], [1] Institute of Microbiology & Immunology, [2] Institute of Genetics, National Yang-Ming Medical College, Taipei, Taiwan, R.O.C.

We isolated five spontaneous ethidium bromide(EtBr)-resistant (*ebr*) mutants of *S. lividans* TK64: YJ11, YJ51, YJ52 and YJ57 were completely resistant to 15 μM of EtBr, and YJ18 was partially sensitive. Genetic mapping by conjugation placed the *ebr* mutations of YJ11, YJ51, YJ52, and YJ57 at about 9 o'clock position of the chromosome and that of YJ18 at about 6 o'clock position. YJ11, YJ51, YJ52, and YJ57 exhibited cross resistance to proflavin and acriflavine, but YJ18 did not. These results suggested that the *ebr* mutations in YJ11, YJ51, YJ52 and YJ57 belong to the same class, and that in YJ18 belongs to another. Furthermore, all the *ebr* mutants were more resistant to acridine orange than TK64, and about as sensitive to adriamycin as TK64. DNA fragments were cloned from YJ11 and YJ51 which conferred *partial* resistance to EtBr. The two cloned sequences (*ebr*-11 and *ebr*-51) shared a 2-3 kb homology which was originated from the same genomic region, but had undergone rearrangements during cloning. No amplification or gross rearrangement of DNA was detected in the *ebr* mutants. The complete resistance exhibited by YJ11 and YJ51 and the partial resistance exhibited by TK64 harboring the cloned *ebr*-11 or *ebr*-51 sequence suggest that *ebr*-11 and *ebr*-51 are blocked in EtBr transport. The possibility that this transport system is also for hydrophobic, branched amino acids is being investigated.

CC 105 **ANALYSIS OF MYCOBACTERIAL BIOSYNTHETIC GENES**, Jeffrey D. Cirillo, Lisa Pane, Barry R. Bloom, and William R. Jacobs, Department of Microbiology & Immunology, Albert Einstein College of Medicine, Bronx, NY 10461. Although mycobacteria are one of the earliest known pathogens of man little is known about the genetics of biosynthetic pathways in these organisms. The study of amino acid and cell wall biosynthesis in mycobacteria should contribute to both the understanding of gene expression in mycobacteria and production of multiple markers for genetic analyses and maintenance of plasmids. We have isolated mycobacterial genes with known functions by complementation of *E. coli* mutations. We screened a genomic library of partially digested *Mycobacterium smegmatis* DNA in λgt11 for the ability to complement the *E. coli gltA*, *aroB*, *trpB*, *thyA*, and *asd* mutations. In the case of *gltA*, *aroB*, and *asd* several positive clones were isolated and restriction mapped. We have used the cloned *M. smegmatis* gene to isolate the Bacille Calmette-Guerin (BCG) *asd* gene by Southern hybridization of a BCG genomic library. We have partially sequenced these genes and have localized the position of the *asd* gene through complementation analysis using a set of nested deletions. Utilizing this information it should be possible to construct a mycobacterial strain with a large deletion in the *asd* gene by gene replacement. An *asd* auxotrophic mutation and the cloned complementing gene should provide a strong selection for stably maintaining recombinant DNA molecules in mycobacterial cells since the *asd* gene is involved in the synthesis of methionine, threonine, lysine, and diaminopimelic acid. Strains constructed in this manner should be ideal for stably maintaining plasmids in a recombinant BCG vaccine (even in animal hosts) and the identification of virulence genes of pathogenic mycobacteria.

CC 106 PHOSPHATIDYLINOSITOL TRANSFER PROTEIN IS ESSENTIAL IN YEAST, William Dowhan and Jacqueline Aitken, Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, Texas 77225. Phospholipid transfer proteins catalyze the movement of phospholipids between a large variety of membranes *in vitro*, and it has been proposed that these proteins participate in intracellular phospholipid movement *in vivo*. However, the true *in vivo* function of these proteins remains unknown. A labeled oligonucleotide based on the amino terminal sequence of the phosphatidylinositol transfer protein (PITP) from *Saccharomyces cerevisiae* was used to screen a yeast genomic library. Plasmids were isolated which when carried in yeast resulted in overproduction of both PITP activity and PITP-specific antigen. The protein sequence predicted from the DNA sequence of *PIT1* gene agrees with the determined N-terminal sequence of purified PITP and suggests the presence of an intron near the 5'-terminus of the gene. Tetrad analysis of a diploid strain in which one copy of the *PIT1* gene had been interrupted demonstrated that PITP is essential for cell viability and growth. Further genetic and biochemical studies are underway to determine the functions of PITP in eukaryotic cells. Supported in part by NIH grant #GM 35143.

CC 107 PHOSPHATE IN PRIMARY AND SECONDARY METABOLISM OF STREPTOMYCES COELICOLOR A3(2). Gary R. Janssen, Joe Liebescheutz, and Timothy Will. Department of Biology, Indiana University, Bloomington, IN 47401.
Phosphate is an essential nutrient and is involved in the structure and function of many biological molecules. The concentration of phosphate has been observed frequently to influence the timing and production levels of many antibiotics in various species of Streptomyces. We have initiated a mutational search for genes of S. coelicolor that are involved in the sensing and recovery of external phosphate. Using a chromogenic indicator (X-P) of phosphatase activity, we have mutationally identified genes involved in the regulated expression of alkaline phosphatase activity. We have also observed that the production of actinorhodin by S. coelicolor is regulated, in part, by phosphate concentration. Using phosphate concentrations that normally repress actinorhodin production, we have mutationally identified genes that relieve phosphate repression of actinorhodin. These mutations and mutations that alter the expression of alkaline phosphatase are being mapped to the S. coelicolor chromosome and efforts are underway to isolate and analyze these genes by molecular cloning techniques.

CC 108 PEPTIDE TRANSPORT IN STREPTOMYCES COELICOLOR A3(2), Michael J. Leigh and David A. Hodgson, Department of Biological Sciences, University of Warwick, COVENTRY, CV4 7AL, United Kingdom.
Streptomyces coelicolor A3(2), the genetically best characterised streptomycete, survives in the soil by the export of degradative enzymes followed by uptake and metabolism of the small products of their reactions. A number of publications have concerned amino-acid transport in Streptomyces spp. but there is no literature on the perhaps ecologically more important role of peptide transport. Peptide and amino-acid transport is of interest as amino-acids are involved in the synthesis of many secondary metabolites.

Mutants isolated in our laboratory during work on amino-acid metabolism were unable to transport proline. Further mutants isolated from this stock were resistant to several antibiotics structurally analagous to di-, tri-, or tetrapeptides. A number of these mutants appear to be resistant to the peptide antibiotics due to impaired efficiency of peptide transport. When inoculated onto silica gel containing proline-peptides these mutants failed to grow whereas the parental strain did grow. Data will be presented which implies Stm coelicolor A3(2) has at least two peptide transport mechanisms with overlapping peptide specificities.

CC 109 CLONING AND CHARACTERIZATION OF THE HISTIDINE BIOSYNTHETIC GENE CLUSTER OF Streptomyces coelicolor, Danila Limauro, Sandra Avitabile, *Melania Cappellano, *Anna Maria Puglia and Carmelo B. Bruni, Centro di Endocrinologia ed Oncologia Sperimentale del C. N. R., Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli, Via S. Pansini 5, 80131 Napoli, and *Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo, Via Archirafi 22, 90123 Palermo, Italy.
Former biochemical and genetic evidences indicate that in Streptomyces coelicolor the majority of the genes involved in the biosynthesis of the amino acid histidine are clustered in a small region of the chromosome and that their expression is regulated. In order to investigate the structural organization and the regulation of these genes we have constructed genomic libraries from S. coelicolor strain A3(2) in the pUC vectors. Recombinant clones were isolated by complementation of an Escherichia coli hisBd auxotrophic strain. A recombinant plasmid containing a 3.4 Kb fragment of genomic DNA was further characterized. This fragment cloned in plasmid vector PJ699 is able to complement hisB S. coelicolor mutants. Overlapping clones spanning a genomic region of 15 Kb were isolated by screening other libraries with the labeled 3.4 Kb fragment. DNA sequence analysis of a 4 Kb region allowed to identify 5 ORF's which show significant homology with the his gene products of E. coli. In particular the order of the genes: 5' hisD, hisC, hisBd, hisH, hisA 3', is the same as in the his operon of E. coli.

CC 110 CLONING OF A GENE GOVERNING LYSINE-ε-AMINOTRANSFERASE, THE FIRST ENZYME IN β-LACTAM BIOSYNTHESIS IN Streptomyces. K. Madduri[1], C. Stuttard[1] and L.C. Vining[2]. Departments of [1]Microbiology and [2]Biology, Dalhousie University, Halifax, NS, Canada, B3H 4H7.
We have previously shown that the pathway generating α-aminoadipate, a precursor for β-lactam antibiotic biosynthesis, is present in β-lactam producing streptomycetes such as Streptomyces clavuligerus and is absent from non-producers such as S. lividans (K. Madduri, C. Stuttard and L.C. Vining, 1989, J. Bacteriol. 171:299-302). Lysine-ε-aminotransferase, the first enzyme in the α-aminoadipate pathway, is not essential for growth, and its regulation is like that of other β-lactam biosynthetic enzymes; this indicates that the enzyme acts specifically in a secondary metabolic pathway. Since genes governing the biosynthesis of individual antibiotics are generally clustered, we decided to use a cloned β-lactam biosynthesis gene specifying Isopenicillin-N synthase (IPNS) to isolate DNA which might include the gene for lysine-ε-aminotransferase. Using the technique of "chromosome walking" we identified overlapping DNA fragments from the genomic library of S. clavuligerus carried in a lambda replacement vector (lambda GEM-11). One DNA fragment was cloned in a shuttle vector created by fusing the E. coli vector, pTZ18R, and the Streptomyces vector, pIJ702. The cloned fragment directed the synthesis of lysine-ε-aminotransferase in S. lividans which normally does not produce this enzyme. Restriction mapping revealed the location of the cloned gene close to the IPNS gene. This location is consistent with lysine-ε-aminotransferase having a specific role in β-lactam biosynthesis in Streptomyces.

CC 111 PHYSIOLOGICAL STUDIES ON THE GROWTH AND THE PRODUCTION OF INSECTICIDAL ANTIBIOTICS OF STREPTOMYCES NANCHANGENSIS, Guoquan Tu, Liang Ouyang, Jiangxi Agricultural University, Nanxhang Jiangxi, the People's Republic of China.
A study was made on the physiology of the growth and production of insecticidal antibiotics of Streptomyces nanchangensis. The favourable environmental conditions and the metabolic changes in fermentation were also tested. The optimum fermentative media and environmental conditions suitable to its growth and production of insecticidal antibiotics were screened out by using orthogonal experiments. Preliminary approach by regression analysis showed that the interrelationship between the growth and production of insecticidal antibiotics of Streptomyces nanchangensis could be expressed in a formula as $\ln(p/x)=\ln a+bx (b<0)$. That is to say the correlation between the growth yields and the natural logarithm of probit of the antibiotic yields appeared negative. A mathematical model of the interrelationship between the growth yields and the probit of antibiotic yields, $p=axe^{bx} (b<0)$, was established.

CC 112 CLONING AND EXPRESSION OF ACYL CARRIER PROTEIN INVOLVED IN FATTY ACID BIOSYNTHESIS IN *Saccharopolyspora erythraea* ,W. Peter Revill and Peter F. Leadlay, Department of Biochemistry, University of Cambridge, Tennis court road, Cambridge, CB2 1QW, England.
A small, discrete acyl carrier protein (ACP), thought to be a component of fatty acid synthase has been purified from *S. erythraea* (1). The gene for this ACP has been cloned and sequenced and shown to encode a polypeptide of 95 amino acid residues (deduced M_r 10423). Analysis of the flanking DNA sequence should now find whether or not at least some of the fatty acid synthase genes are clustered in this region. This ACP gene has been efficiently expressed in *Escherichia coli* using the pT7-7 system (2, 3). The protein is soluble and constitutes, after induction, at least 10% of total cell protein. The ability to express such genes in *E. coli* should greatly facilitate the structural and functional characterisation of the gene products.
1. Hale R.S. *et al* (1987). *FEBS lett.* **224**: 133-136
2. Tabor S. and Richardson C. C. (1985). *Proc. Natl. Acad. Sci.* USA **82**:1074-1078
3. Swan D. G. *et al* (1989). *J. Bacteriol.* (In press).

CC 113 GENETIC SUPPRESSION OF DEFECTIVE *melC1* GENE OF *STREPTOMYCES ANTIBIOTICUS* BY AN *ompR*-LIKE GENE OF *STREPTOMYCES LIVIDANS*. Huei-Chung Tseng[1,3], Carton W. Chen[1,2] [1] Institute of Microbiology & Immunology, [2] Institute of Genetics, National Yang-Ming Medical College; [3] Department of Microbiology, Soochow University, Taipei, Taiwan, R.O.C.
The melanin (*melC*) operon of *S. antibioticus* contains the tyrosinase (*melC2*) gene and a upstream *melC1* gene. The MelC1 protein is essential for the expression of tyrosinase and has been postulated to be involved in the transfer of copper to apotyrosinase. We generated and identified two mutations in the cloned *melC1* gene on pIJ702: pLUS130 contained a His-117 to Tyr-117 substitution, and pLUS132 contained an AU*G* to AU*A* transition at the initiation codon. A slow-growing mutant, HT32, of *S. lividans* TK64 was obtained by MNNG mutagenesis that suppressed the Mel$^-$ phenotype of pLUS130 and pLUS132. A 1,685-bp *Pst*I fragment from HT32 was cloned that conferred the suppressor activity, which was narrowed down to a 228-aa ORF (designated CutR) within the sequence. The derived aa sequence of CutR shared homology with OmpR of *E. coli* and related regulatory proteins of the sensor-regulator family. Furthermore, a 133-aa ORF (designated CutS) downstream from CutR displays the characteristics for a multimeric transmembrane protein. We postulate that CutR acts as a regulator for the biosynthesis of membrane proteins of a copper transport system, and that the suppressor mutation in *cutR* results in excessive copper uptake, complementing the defect in the *melC1* gene while causing retarded growth.

CC 114 THE PURIFICATION, CHARACTERISATION AND OLIGONUCLEOTIDE SCREENING OF DAHP SYNTHASE FROM STREPTOMYCES COELICOLOR, Graeme E. Walker,John R. Coggins,Iain S. Hunter and Hugh G. Nimmo, Departments of Biochemistry and Genetics, University of Glasgow, Glasgow G12 8QQ, Scotland. 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase from *Streptomyces coelicolor* has been purified to homogeneity and its regulatory properties studied.No DAHP synthase isozymes were detected. The procedure consisted of salt fractionation, two anion exchange chromatography steps and a hydrophobic interaction chromatography step, resulting in a 1500 fold purification. The molecular weight of the native enzyme and its single subunit species were estimated to be 107 and 54 kDa, respectively, suggesting that the enzyme is a dimer.The effects of pathway end products tryptophan, phenylalanine and tyrosine on enzyme activity were investigated. Only tryptophan elicitted a significant effect, with 125uM capable of a maximal inhibition of 67% of control activity. Direct sequencing of the purified enzyme failed, indicating that the N-terminus was blocked or that it was inaccesible. A second sample was digested with the protease clostripain, peptides purified by reverse phase HPLC, sequences obtained, and used to construct oligonucleotide probes. Screening of digested genomic DNA with this probe has begun. Hybridisation conditions have been optimised to yield strong hybridisation signals, however washing conditions which reveal a unique signal remain to be developed . Following the optimisation of washing conditions we hope to proceed to purify and clone a suitably sized restriction fragment.

CC 115 CHARACTERIZATION OF A PLASMID ENCODED ACTINOMYCETE LIGNIN PEROXIDASE GENE SEQUENCE IN DIFFERENT STREPTOMYCES BY USE OF PROTOPLAST TRANSFORMATION AND CONJUGATIVE MOBILIZATION, Zemin Wang, Bruce H. Bleakley and Don L. Crawford, Department of Bacteriology and Biochemistry, University of Idaho. Moscow ID 83843. The Streptomyces plasmid pIL702.LP, containing a cloned lignin peroxidase gene sequence from the genome of Streptomyces viridosporus T7A, was transformed into several lignin peroxidase-negative Streptomyces viridosporus T7A mutants. Mutants with and without plasmid pIJ702.LP were then grown in solid state fermentations on lignocellulose. These cultures were assayed for extracellular protein and peroxidase production using SDS-PAGE gel and native PAGE gel electrophoresis to help determine whether the cloned sequence encoded the lignin peroxidase gene itself or a regulatory region of the gene. Plasmid pIJ702.LP was also mobilized, by bi- or tri-parental conjugative crosses, from S.lividans strains TK64.1, TK24.1, and TK23.1, into Streptomyces strains negative for lignin peroxidase. Transconjugants were isolated and then characterized as described above using solid state fermentations and PAGE gel analyses. The nature of the cloned lignin-peroxidase gene sequence, as revealed by this studies, is dicussed.

CC 200 INHIBITION OF rRNA TRANSCRIPTION IN STREPTOMYCES COELICOLOR A3(2) ON INDUCTION OF THE TEMPERATE BACTERIOPHAGE ØC31
Tim M. Clayton and Mervyn J. Bibb, John Innes Institute, NORWICH NR4 7UH, UK.
The effect of lytic growth of the temperate phage ØC31 on rRNA transcription in Streptomyces coelicolor A3(2) has been studied. A thermoinducible lysogen of ØC31 (ØC31c^{ts}1) was used to obtain synchronous phage development. Prophage induction severely reduced (about 35-fold) host rRNA transcription when compared to appropriately treated controls. The rrnD gene set of Streptomyces coelicolor A3(2) has four identified that appear to fall into two different classes. High resolution nuclease S_1 mapping showed that these promoters were all affected equally upon prophage induction. Using chloramphenicol as an inhibitor of translation, it was shown that protein synthesis was absolutely required for transcriptional shutoff to occur. These results suggest that a ØC31-specified protein(s) elicits shutoff of host rRNA synthesis during prophage development. Experiments are underway to elucidate the mechanism by which this occurs.

CC 201 *STREPTOMYCES* β-LACTAMASE PROMOTERS: TRANSCRIPTIONAL INDUCTION AND MUTAGENIC ANALYSIS OF FUNCTIONAL MOTIFS, Mats Forsman, Lena Lindgren, and Bengtåke Jaurin, Department of Cell and Microbiology, National Defence Research Establishment, S-901 82 Umeå, Sweden. The β-lactamase expression in *Streptomyces cacaoi* could be induced by the β-lactam compound 6-amino penicillinoic acid (6-APA). In liquid cultures, a 50-fold increase of β-lactamase expression was observed within the first three hours after addition of 6-APA. Using the cloned β-lactamase gene as a probe, it was shown that this increase was mediated at the level of transcriptional initiation. The start point of the induced β-lactamase transcript was determined, and the nucleotide sequence of the promoter region was analyzed. No noticeable homology was found to control regions of inducible β-lactamase genes of other bacteria. A striking feature was the presence of six direct repeats (ten base pairs each) upstream of the promoter region. Thus, an example of an inducible regulatory gene system in *Streptomyces* is presented.

The promoter for the β-lactamase gene of *S. fradiae* was localized by primer extension analysis and characterized. To define functional motifs of the *S. fradiae* β-lactamase promoter region, an extensive site-specific mutagenic analysis was performed. The effect on transcription by these promoter mutations will be discussed.

CC 202 IDENTIFICATION OF TRANS-ACTING MUTATIONS THAT AFFECT REGULATION OF THE *STREPTOMYCES galP1* PROMOTER. C. Ingram[1], M. Brawner[2] and J. Westpheling[1]. University of Pennsylvania Medical School, Philadelphia,[1] and SmithKline Beecham Laboratories, King of Prussia. [2]

The *galP1* promoter, located at the 5' end of the *Streptomyces gal* operon, is responsible for glucose-sensitive, galactose-dependent transcription of the galactose utilization structural genes. We have transformed mutagenized cells of *S. coelicolor* with an SCP2 plasmid derivative containing a wildtype *galP1-xylE* transcriptional fusion and screened for mutations that affect *galP1* regulation. Colonies were identified that expressed catechol dioxygenase after growth on glucose or on a neutral carbon source (glycerol) in the absence of galactose. These results were confirmed using RNA dot blot assays that detected transcription from the chromosomal copy of *galP1* in strains cured of the SCP2 *galP1-xylE* fusion plasmid. We are now characterizing these mutations and attempting to clone the genes they identify.

CC 203 ANALYSIS OF THE PROMOTER REGION OF THE MELANIN LOCUS FROM STREPTOMYCES ANTIBIOTICUS, Yan-Hwa Wu Lee, Wei-Ming Leu, Shwu-Yuan Wu, Szecheng J. Lo, and Jin-Jer Lin, Institute of Biochemistry, Microbiology and Immunology, Natl. Yang-Ming Med. College, Taipei, Taiwan 11221, Republic of China.
Several approaches were used to study the transcriptional control region of the melanin-production locus (melC) of Streptomyces antibioticus. Filter-binding in combination with exonuclease III protection localized the 3' boundary of a Streptomyces RNA polymerase binding site predominantly about 39 nt upstream of the start codon of melC1, the first open reading frame in the melC locus. Deletion of nucleotide(nt) 112 to 197 upstream of the melC1 start codon reduced melC expression to less than 10%, and deletion of nt 28 to 107 or 28 to 120 upstream of melC1 totally inactivated melC. High-resolution nuclease S1 mapping identified the in vivo transcriptional start point (tsp) at 33-34 nt upstream of the start codon of melC1. No sequence resembling the E. coli consensus promoter sequence was found in this region, and site-directed mutagenesis of such a sequence located 101 to 132 nt upstream of melC1 did not inflence melC expression. These studies suggest that transcription of melC is principally from a single tsp and is positively regulated by a mechanism that involves DNA sequences 87-163 nt upstream of the tsp (Supported by NSC-77-0412-B010-23 and NSC-78-0412-B010-01 from NSC, R.O.C.).

CC 204 MUTATIONAL ANALYSIS OF THE *STREPTOMYCES galP1* PROMOTER. S.Mattern[1], J. Fornwald[2], M. Brawner[2] and J. Westpheling[1]. University of Pennsylvania Medical School, Philadelpia,[1] and SmithKline Beecham Laboratories, King of Prussia.[2]

A promoter, *galP1*, located at the 5' end of the *Streptomyces gal* operon, is responsible for glucose-sensitive, galactose-dependent transcription of the galactose utilization structural genes. A mutational analysis of the DNA sequence upstream of the apparent transcription start site of *galP1* has revealed surprising and unusual features of this catabolite controlled promoter. Using oligonucleotide-directed mutagenesis, we have introduced mutations within *galP1* that affect promoter function and regulation. The effects of these mutations were analyzed by making transcriptional fusions to the *Pseudomonas putida xylE* gene and measuring the activity of catechol dioxygenase. There are two striking features of the DNA sequence of *galP1* which we have shown to be important to promoter function and regulation: six consecutive guanidyl residues centered around -35 and a series of hexamers that conform to the consensus TGTGAT. Single base changes within the series of G residues either greatly increase expression from *galP1* or eliminate it entirely. Certain changes within the TGTGAT hexamers result in expression from *galP1* in the absence of inducer. Our observations suggest that these hexamers constitute an operator that may bind repressor in a cooperative fashion. We are continuing to analyze these *cis*-acting regulatory elements using site-directed mutagenesis.

CC 205 TRANSCRIPTIONAL ANALYSIS OF DNA INVOLVED IN SPORULATION OF *STREPTOMYCES GRISEUS*, Lee A. McCue, Martin J. Babcock, and Kathleen E. Kendrick, Department of Microbiology, The Ohio State University, Columbus, OH 43210
A region of DNA that restores sporulation to *Streptomyces griseus* bald mutants was analyzed for *in vivo* promoter activity, transcript size, and changes in transcription during vegetative growth and differentiation. Two promoter-active sites alternate with two overlapping open reading frames that have the potential to encode polypeptides of 55,500 and 49,500 daltons. The downstream transcript, prevalent during vegetative growth, decreased in relative abundance more than 40-fold during the first four hours of submerged sporulation. Transcriptional fusions to the *xylE* gene of *Pseudomonas putida* also demonstrated a relative decrease in gene expression upon the induction of sporulation by phosphate starvation. Analysis of transcript length in *E. coli* and *S. griseus* suggested that this coding region is monocistronic. To determine the number of polypeptides encoded by this DNA, we have initiated site-directed mutagenesis of both the N-terminal region of the longer open reading frame and the in-frame TTA midway through the coding sequence. Sequencing of the analogous DNA (>90 % similarity) from *Streptomyces coelicolor* is in progress. The results of these experiments will be reported.

CC 206 THE OXYTETRACYCLINE GENE CLUSTER OF S. RIMOSUS: SEQUENCE OF OTCZ AND TRANSCRIPTION PATTERN AT 'LEFT' END OF CLUSTER. Kenneth J. McDowall and Iain S. Hunter, Institute of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS, UK.
Streptomyces rimosus produces the polyketide antibiotic oxytetracycline (OTC). All the biosynthetic genes are located in a single cluster, flanked by two resistance genes [Binnie et al. (1989) J. Bacteriol. 171, 887-895]. The otrA resistance gene protects the ribosome from arrest of translation by OTC, while otrB results in decreased accumulation (increased efflux) of the drug from the cell [Ohnuki et al. (1985) J. Bacteriol. 161(3), 1010-1016]. Two transcription start sites exclusive for the otrA gene have been identified; otrAp1 and 2 respectively [Doyle et al. (1988) Heredity 61, 305]. The relative abundance of transcripts from otrAp1 and otrAp2 changes across an antibiotic fermentation, as determined by high resolution S1 mapping at the 5' end. Furthermore, the otrA gene appears to be co-transcribed with the otcC (anhydrotetracycline oxygenase) and otcZ genes as part of a polycistronic message. The otcZ gene has recently been sequenced and has the potential to encode a protein of 344 amino acids and M.W. 37KD. As yet no striking homologies have been found.

CC 207 CHARACTERIZATION OF THE *S.GRISEOLUS* SULFONYLUREA INDUCIBLE CYTOCHROMES P450 OPERONS , Nina V. Patel, Charles A. Omer, Patricia J. Litle. Central Research and Development Department, E.I. DuPont, Experimental Station, Wilmington, DE 19800-173. Two classes of sulfonylurea modifying inducible cytochromes P450 are found in *S. griseolus* : P450SU1 and P450SU2. P450 SU1 and SU2 are the products of the *suaC* and *subC* genes respectively. The genes are organized within two operons, each containing the P450 gene and its corresponding ferridoxin gene: *suaC,B* and *subC,B*. Expression of these operons in *S. griseolus* is transcriptionally regulated, but the two P450 transcripts are regulated independent of one another. Primer extension analysis was preformed to determine the trancriptional start sites. Sequence analysis of the *suaP* and *sub* P1,P2 promoters show that they belong to a class of Streptomyces E.coli-like promoters(SEP). Inverted repeats are present in the P450 transcripts, either in the promoter region or immediately downstream of the transcriptional start site. We have identified DNA binding proteins that bind to these inverted repeat sequences and are currently examining their involvement in gene regulation.

CC 208 CHANGES IN THE RIBOSOMAL PROTEIN PATTERN DURING DIFFERENTIATION IN *Streptomyces antibioticus*. J.A.Salas, F.Parra, L.M.Quirós, C.López Otin, C.Méndez and C.Hardisson,Departamento de Biologia Funcional,Universidad de Oviedo, 33006 Oviedo, Spain. We have detected changes in the ribosomal protein pattern of *S. antibioticus* during differentiation. Some proteins were present in the ribosomes of the mycelium and absent in the spore ribosomes. Functional variations among the different types of ribosomes were also detected, the activity of the spore ribosomes being particularly decreased. One of the proteins lacking in the 50S spore subunit was purified by HPLC and its amino termini sequenced. Computer analysis revealed homology between this protein and the L7/L12 protein. An oligonucleotide mixture was used as probe for the screening of a *S. antibioticus* gene library in the cosmid cos4. Three different cosmid clones were found to contain a 1.5 kb BamHI fragment that hybridized with the probe, whose nucleotide sequence is now being determined.

CC 209 REGULATION OF THE GLYCEROL OPERON OF STREPTOMYCES COELICOLOR, Colin P.Smith, Department of Biochemistry and Applied Molecular Biology, UMIST, PO BOX 88, Manchester, M60 1QD, UK. The pathway for glycerol catabolism in *Streptomyces coelicolor* is determined by the glycerol (*glyIABX*) operon. The structure and transcriptional regulation of *glyIABX* and its associated specific activator gene, *glyIR*, have been analysed in detail (C.P.Smith and K.F.Chater (1988) *Mol. Gen. Genet.*, 211, 129-137; (1988) *J.Mol.Biol.*, 204, 569-580). The above work will be summarised and more recent studies on transcriptional regulation of *glyIABX* in wild-type and mutant strains will be presented. This will include: (i) Evidence for the existence of a transcription "antitermination" system within *glyIABX*; (ii) Studies of a pleiotropic mutant defective in *glyIABX* promoter utilisation, "antitermination" and glucose repression of *glyIABX*; (iii) Current progress with site-directed mutagenesis of *glyIR*, the tandem *glyIABX* promoters, and potential Z-DNA forming sequences; (iv) Preliminary studies on the influence of genetic location on *glyIABX* expression.

CC 210 TRANSCRIPTION OF THE ØC31 REPRESSOR GENE: MULTIPLE INTERNAL 5' ENDPOINTS. Maggie Smith, Institute of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS, Scotland. Clear plaque mutants of the *Streptomyces* temperate phage ØC31 map to a single gene, *c*, which has been cloned, sequenced and expression predicted of a protein containing a helix-turn-helix DNA binding motif towards the C-terminus (Sinclair and Bibb, 1988 MGG : 213 : 269-277). High resolution S1 mapping at the promoter, *p*1, and at the terminator t_1 suggests the synthesis of a transcript of approximately 2100nt (Sinclair and Bibb, personal communication). Low resolution S1 mapping now shows that in addition to the predicted transcript, mRNAs are detected with their 5' endpoints internal to the *c* gene (Fig.1). There is also a second terminator, t_2 (Fig 1). Induction by heat shock of a *cts* lysogen results in a 50 to 100 fold increase in abundance of the mRNAs terminating at t_1 and a further transcript (600nt) is detected which probably initiates upstream of the SphIG fragment and terminates at t_3. Primer extension of mRNA preparations have mapped the internal 5'-endpoints. Downstream of each endpoint is a possible initiation codon with sequences similar to a *Streptomycete* ribosome binding site.

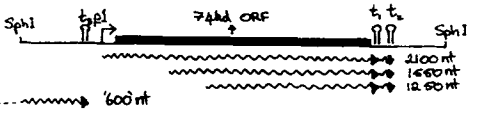

Figure 1 Transcription map of the SphIG fragment of ØC31. Wavy lines represent mRNAs. Numbers represent mRNA length in nucleotides (nt) to terminator, t_1.

Antibiotic Biosynthesis and Resistance

CC 300 CLONING THE PATHWAY OF MONENSIN BIOSYNTHESIS, Teresa J. Arrowsmith[1], Paco Malpartida[1], David H. Sherman[1], David A. Hopwood[1] and John A. Robinson[2], [1]John Innes Institute, Norwich, NR4 7UH, UK and [2]Organisch-Chemisches Institut, Universität Zürich, CH-8057 Zürich, Winterthurerstrasse 190, Switzerland.
Streptomyces cinnamonensis produces the polyether antibiotic monensin-A. DNA which may encode enzymes involved in the monensin-A biosynthetic pathway has been isolated from an EMBL4 library of S.cinnamonensis chromosomal DNA. The library was probed with actI and actIII, whose gene products operate early in the actinorhodin pathway of S.coelicolor and encode components of the actinorhodin polyketide synthase. Work is in progress to complete the sequence of the actI, actIII homologous DNA, which also shows sequence homology to genes that encode Streptomyces acyl carrier proteins. The EMBL4 clone has been subcloned as four BamHI fragments in the Streptomyces vector pWOR125; these subclones are being used in complementation studies with Streptomyces cinnamonensis mutants blocked in monensin-A biosynthesis.

CC 301 A POLYKETIDE SYNTHASE GENE CLUSTER FROM *Streptomyces curacoi*: CLONING AND EXPRESSION. Staffan Bergh and Mathias Uhlén, Department of Biochemistry and Biochemical Technology, Royal Institute of Technology, S-100 44 Stockholm, Sweden.

The polyketides from various streptomycetes are interesting both on account of their pharmaceutical/industrial importance and because of the similarity, but non-identity, of their biosynthesis to that of the fatty acids.
We have used the previously cloned *actI* gene (Malpartida and Hopwood, Nature, 309 (1984) 462-4) to probe a library of *S.curacoi* DNA fragments for the curamycin synthase genes. Curamycin is a relatively simple polyketide built up from only 4 acetate units without reductive or other steps.
The sequencing data reveals that the clones contain at least the two open reading frames associated with other cloned streptomycete polyketide synthases. Constructions with the putative curamycin synthase genes under control from a vector promoter, transformed to *S. curacoi*, confers abundant production of a brown pigment.

CC 302 CLONING GENES FROM STREPTOMYCES TENDAE INVOLVED IN NIKKOMYCIN BIOSYNTHESIS, Christiane Bormann, Karin Aberle, Sibylle Mattern*, Hildgund Schrempf*, Department of Microbial Genetics, University of Tübingen, 7400 Tübingen, *University of Osnabrück, FRG.
Nikkomycins belong to the nucleoside peptide antibiotics and exhibit a high antifungal, insecticidal and acaricidal activity. Their specific target is chitin biosynthesis acting as competitive inhibitors of chitin synthetase. S. tendae Tü901 produces a spectrum of various nikkomycins. Main components (1g/l) are nikkomycin Z and X., the most efficient inhibitors. After UV and NTG mutagenesis we isolated non-producing mutants as well as mutants blocked in the biosynthesis of the imidazolone base occuring in the nikkomycin X-series and of the amino acid, nikkomycin D. In shot-gun cloning experiments using pIJ699 as vector a 9,4 kb DNA fragment was isolated from S. tendae which complemented the non-producing mutant NP9 to the formation of the inactive nucleoside structures, nikomycins C/C_X (160 mg/l) and the biologically active nikkomycin K_X (20-30 mg/l). In Southern hybridization experiments the cloned DNA exclusively reacted with S. tendae DNA sequences. As shown by Northern dot blotting transcripts of the isolated DNA fragment were only detected during stationary growth and correlated with the extent of nikkomycin production. When the recombinant plasmid pNP113 containing the 9,4 kb DNA fragment was transferred into the over-producing mutant S2566, transformants exhibited a significantly decreased capacity for forming nikkomycin. Southern analyses of genomic DNA of these transformants revealed that severe rearrangements occured in DNA sequences being homologous to the 9,4 kb insert of pNP113. The cloned DNA was used as probe for a gene bank of S. tendae to isolate other genes required for nikkomycin production.

CC 303 BIOSYNTHESIS OF ANTITUMOR ANTIBIOTIC REBECCAMYCIN AND DIFFERENTIATION OF THE PRODUCING ACTINOMYCETE. J. A. Bush, R. L. Berry and S. Forenza, Bristol-Myers Company, Antitumor Chemistry and Microbiology Department, Pharmaceutical Research and Development Division, P.O. Box 5100, Wallingford, CT 06492. Rebeccamycin, a chlorinated indolocarbazole antitumor agent, is produced by *Saccharothrix aerocolonigenes* strain C38383-RK2. Five isolates without aerial mycelium were selected from C38383-RK2 and these isolates gave scant production of rebeccamycin. On the other hand isolates with aerial mycelium show substantial production of this activity. Similarly it was reported with *Streptomyces coelicolor* strain A3(2) that mutations in the genes required for aerial mycelium formation abolish antibiotic production. Rebeccamycin, isolated from the mycelium, inhibits the growth of mammalian cell lines and produces single strand breaks in DNA of human cell line A549. Rebeccamycin, staurosporine, K252A and AT2433 are related actinomycete secondary metabolites. Staurosporine is an inducer of differentiation in human neuroblastoma cell line NB-1 and K252a is an inhibitor of protein kinase C. Strain C38383-RK2 grows rapidly on minimal medium and *Nocardiopsis* sp. strain K-252, NRRL 15532, producer of K252a, is a slow growing strain showing aerial mycelium on some agar media with scant growth on minimal medium. Isolated from C38383-RK2, mutant strain C38383-R32 failed to produce aerial mycelium with subculture on diverse media and gave traces of rebeccamycin production. Since members of the genera *Saccharothrix* and *Nocardiopsis* are closely related, strain C38383-R32 and strain K252 were selected for protoplast fusion experiments. Isolate H9, derived from protoplast fusion studies, gave rapid growth on minimal medium, aerial mycelium on agar media and restored rebeccamycin type activity in flask culture.

CC 304 THE BIOSYNTHETIC ORIGIN OF THE PYRIDONE RING OF EFROTOMYCIN, Gary Darland and Lou Kaplan, Merck and Co., Inc., P. O. Box 2000, Rahway, NJ 07065.

Efrotomycin is a representative of the elfamycin class of antibiotics synthesized by *Nocardia lactamdurans* . The molecule consists, in part of a polyketide chain linked to N-methyl-4-hydroxy pyridone. While the precursors of the polyketide have long been known, the origin of the pyridone ring has remained elusive. Derepression of the pyrimidine biosynthetic pathway, with concomitant uracil excretion, has been previously shown to correlate with an increased capacity for efrotomycin biosynthesis. The efficient incorporation of [5,6-^{3}H]- uracil into efrotomycin has now been demonstrated. In addition, the reductive uracil catabolic pathway has been found using both whole cells and crude extracts. Taken together these observations suggest that the pyridone ring may originate from ß-alanine derived from the reductive catabolism of uracil. This conjecture has been confirmed by demonstrating the incorporation of [4,5-^{13}C]-uracil into carbons 4 and 5 of the pyridone ring.

CC 305 TEICOPLANIN RESISTANCE IN ACTINOPLANES TEICHOMYCETICUS, M.Denaro, M. Sosio and R.Lorenzetti, Lepetit Research Center, M.D.R.I., Via R.Lepetit 34, 21040 Gerenzano (VA),Italy.

The resistance mechanism present in A. teichomyceticus, the producing microorganism of the antibiotic teicoplanin was studied and compared with the emerging resistant phenotypes by gene cloning using S. lividans as host. Teicoplanin is a glycopeptide antibiotic, produced by Actinoplanes teichomyceticus, structurally related to vancomycin. This new antibiotic, recently introduced in clinical practice, is active against gram positive bacteria and is useful in the treatment of severe infections caused by these microorganisms. Its mechanism of action consists in blocking the cell wall biosynthesis by binding to the amino-acyl D-alanyl-D-alanine with the consequent inhibition of the peptidoglycan polymerization. Plasmid mediated resistance to teicoplanin and vancomycin has been described in clinical isolates of enterococci; however the biochemical mechanism/s of resistance have not yet been determined.

We have found that A. teichomyceticus possesses at least one gene conferring teicoplanin resistant character; the mechanism of this resistance has been shown to be due to an altered contact between the drug and its target.

The deduced aminoacid sequence of the protein involved will be discussed and related to the evolution of resistance in pathogenic microorganisms.

CC 306 ORGANIZATION OF THE GENES REQUIRED FOR 6-DEOXYERYTHRONOLIDE B FORMATION IN Saccharopolyspora erythraea Stefano Donadio, Michael Staver, James Tuan and Leonard Katz, Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60048

Erythromycin (Er) is a macrolide antibiotic produced by Saccharopolyspora erythraea through a complex biochemical pathway whose schematic events are: formation of a 14-member macrolactone ring; formation and attachment of a mycarose unit to the ring; and formation and attachment of a desosamine unit to the ring. This pathway has been only partially elucidated by analysis of blocked mutants and characterization of Er intermediates. The first step in the biosynthetic pathway, formation of 6-deoxyerythronolide B (6dEB), is believed to occur through the condensation of seven 3-carbon precursors by an enzymic system similar in function to fatty acid synthase. eryA genes, required for the formation of 6dEB, are clustered around ermE, the gene conferring resistance to erythromycin. Other ery genes near ermE have been identified. eryA genes were initially isolated by their ability to restore Er production when introduced into eryA mutants. Nucleotide sequence and gene disruption experiments indicate that greater than 15 kb are required for 6dEB formation. The eryA genes appear to consist of a 5 kb module, containing acyl carrier protein-, condensing enzyme-, acyltransferase- and reductase-like domains, present as tandem repeats that shares extensive homology with each other and with fatty acid synthase moieties. This complex organization suggests that the eryA genes arose by duplication of a fatty acid synthase-like module.

CC 307 CLONING OF MIDECAMYCIN RESISTANCE GENES FROM *STREPTOMYCES MYCAROFACIENS*, *STREPTOMYCES LIVIDANS* AND *STREPTOMYCES COELICOLOR*, Osamu Hara *† and C. Richard Hutchinson, *School of Pharmacy and Department of Bacteriology, University of Wisconsin, Madison, WI 53706; †Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama, 222 Japan.

DNA conferring resistance to midecamycin A_1, a 16-membered macrolide antibiotic, was cloned from the producing organism, *Streptomyces mycarofaciens* ATCC21454 (*mdmA* gene), and from *Streptomyces lividans* 66 (*lrm* gene) and *Streptomyces coelicolor* M110. The phenotype imparted to *S. lividans* and *Streptomyces griseofuscus* transformants by the cloned DNA seqments indicates that they encode an MLS-type of resistance activity that is inducible by midecamycin and some other macrolides. This has been independently verified by Jenkins and Cundliffe through their studies of the inducible ribosomal RNA methylase activity encoded by the *lrm* gene (E. Cundliffe, personal communication). The *mdmA* and *lrm* genes could be distinguished by the phenotypes of their respective *S. lividans* and *S. griseofuscus* transformants, whereas the *S. lividans lrm* and *S. coelicolor* MLS genes appear to be identical on the basis of their restriction maps and behavior in *S. lividans* and *S. griseofuscus* transformants. DNA fragments containing the *mdmA* and *lrm* genes exhibited bidirectional promoter activity when they were cloned in pIJ486/7 and the corresponding *S. lividans* transformants were assayed for neomycin resistance. *S. mycarofaciens* contains only a single midecamycin resistance gene, which is located near a midecamycin biosynthesis gene. In contrast, *Streptomyces thermotolerans*, which produces carbomycin, a macrolide antibiotic with a very similar structure to midecamycin A_1, has been shown by researchers at Eli Lilly & Company to contain two carbomycin resistance genes, one of which is adjacent to the *carE* gene that appears to encode an isovalerylCoA transferase involved in carbomycin biosynthesis.

CC 308 METHYLENOMYCIN PRODUCTION IN STREPTOMYCES COELICOLOR: OPTIMAL PRODUCTION IN DEFINED MINIMAL MEDIA, Glynn Hobbs, J. Clark Mason, Anthony I.C. Obany, David C.J. Gardner, & Stephen G. Oliver, Biotechnology Centre & Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester, M60 1QD, U.K. The antibiotic methylenomycin A is a plasmid encoded antibiotic produced by Streptomyces coelicolor A3(2). We shall report on a simple defined minimal medium that has been formulated to result in optimal production of the metabolite. The effects that the nitrogen and carbon source (that is presence or absence of glucose and type of amino acid) has on the production of methylenomycin and its derivative (desepoxy methylenomycin) have been examined. By correlating physiological studies of batch and continuous cultures with the quantitative measurement of the antibiotic, we have examined their interrelationship during the "switch" from primary to secondary metabolism the controlling feature of antibiotic production. At the same time we have detected two proteins produced coincidently with methylenomycin production. Using a "reverse genetics" approach we hope to define the genes responsible for their production and so move closer to an understanding of the switch on of this metabolite.

CC 309 GLOBAL CHANGES IN GENE EXPRESSION RELATED TO ANTIBIOTIC BIOSYNTHESIS IN STREPTOMYCES HYGROSCOPICUS, T. Holt, A. Raibaud, C. Laurent, C. Chang*, P. Meyers*, T. Murakami**, E. Takano**, J. Davies, and C. Thompson, Institut Pasteur, Paris, *Cold Spring Harbor Laboratory, New York, **Meiji Seika Kaisha, Tokyo

We are studying the biosynthesis of the antibiotic bialaphos in Streptomyces hygroscopicus as a model system to investigate genetic and regulatory aspects of secondary metabolism. The steps in the biosynthetic pathway of bialaphos and the corresponding biosynthetic genes (bap) have been well-defined. We are now focusing on the more fundamental aspects of the developmental process leading to antibiotic production during stationary growth phase. Expression of these genes is dependent on a regulatory gene (brpA) and shows a temporal correlation with the stringent response.

Computer-assisted analysis of high resolution two dimensional (2D) gel electrophoresis is being used to study global changes in gene expression throughout the growth cycle. Over 20 proteins have been identified as bap gene products since they are not expressed in a regulatory mutant (brpA$^-$). Using cultures pulse-labeled with ^{35}S-methionine at two-hour intervals and analyzed on 2D gels, we have examined the overall pattern of gene expression from early log-phase growth to late stationary-phase growth. Quantitative analyses of time-course experiments indicate that the bap proteins fall into at least five coordinately-regulated families. Genes which encode these proteins can be mapped using the thiostrepton-inducible tipA promoter (Murakami, T., T. G. Holt, and C. J. Thompson. 1989. J. Bacteriol. 171:1459-1466). When expression of a 10-kb fragment of the bap cluster was induced in S. lividans and analyzed on 2D gels, eight new proteins were observed which corresponded to previously observed brpA-specific gene products.

CC 310 INDUCIBLE LINCOMYCIN RESISTANCE IN *Streptomyces lividans*, Gail Jenkins and Eric Cundliffe, Department of Biochemistry. University of Leicester. England.

Streptomyces lividans possesses inducible ribosomal RNA methylase activity that confers high level resistance to lincomycin and lower levels of resistance to certain macrolide antibiotics. The methylase gene *lrm*, is inducible by erythromycin and to a lesser extent by other macrolides. The lincosamide antibiotic celesticetin also induces resistance, while lincomycin itself does not. Here, we show that the *lrm* product monomethylates the N^6-amino group of adenosine at position 2058 within 23S-like ribosomal RNA.

From a mutant of *S. lividans*, constitutively resistant to lincomycin, a 3.5kb DNA fragment has been isolated, cloned in *S. lividans* TK21, and shown to confer constitutively the resistance phenotype that is normally inducible in this strain. Subcloning generated a 1.3kb DNA fragment that still contained *lrm* but conferred a phenotype somewhat different from that of the original clone, in that high level lincomycin resistance was maintained, but resistance to macrolides was markedly reduced.

We therefore suspect that *S. lividans* harbours at least two erythromycin-inducible resistance genes, and that they are both contained within the 3.5kb DNA fragment initially cloned.

CC 311 AMPLIFICATION OF A GENE INVOLVED IN ANTIBIOTIC SYNTHESIS IN STREPTOMYCES USING POLYMERASE CHAIN REACTION, Ralph Kirby and Nancy Dorrat, Department of Microbiology, Upper Campus, University of Cape Town, Rondebosch 7700, R.S.A.

Comparative analysis of the DNA sequences of a number of genes involved in antibiotic synthesis in Streptomyces identified 20mer primers suitable for use in a Polymerase Chain Reaction System. A wide range of species, both producers and non producers were screened using the standard Perkin-Elmer/Cetus conditions and kit using a hybridisation temperature of 40°C, and extension temperature of 72°C and a denaturation temperature of 92°C. Amplified products of the correct size were detected in a number of species. The importance of these results to Antibiotic Screening Programs, Genetic Manipulation of Product Pathways, Streptomyces Taxonomy and Studies on the Evolution of Antibiotic pathways will be discussed.

CC 312 PHYSIOLOGICAL CONTROLS OF EXPRESSION OF STREPTOMYCIN GENES FROM STREPTOMYCES GRISEUS, Helen K. Lindley, Simon Baumberg, Reza Mohamed Fazeli[1] and Jonathan H Cove[1], Department of Genetics and Microbiology[1], University of Leeds, Leeds LS2 9JT, U.K.
We are using the streptomycin synthesis/resistance system as a model to study mechanisms whereby secondary metabolite gene expression is affected by physiological and environmental variables and parameters. We have followed extracellular streptomycin accumulation and the levels of biosynthetic enzyme ADT and resistance enzyme SPH, during growth in various media. In S. griseus batch cultures at 30°C, streptomycin accumulation accompanies growth in minimal medium but is delayed (showing "secondary metabolism" kinetics) in complex medium. Growth of cultures on a dialysis membrane on solid media showed that similar effects applied with minimal vs. complex solid media too. At 34°C, where growth rate was c. 50% that at 30°C, neither streptomycin nor ADT was synthesized, but SPH levels were much as at 30°C. Of the inorganic ions tested, PO_4^{3-}, Mg^{2+} and Ca^{2+}, Mg^{2+} showed a strong inhibitory effect, streptomycin accumulation falling to zero at 0.1 M although growth was unaffected. Continuous culture in minimal medium led to loss of streptomycin formation. As an approach to identification of DNA regions involved in response to physiological state, we have inserted a promoter for strB (encloding ADT) upstream of reporter genes aph and xylE in pIJ702- and SCP2*-based shuttle promoter-probe vectors respectively, and are studying in S. lividans how expression of the reporter gene is affected by conditions of growth.

CC 313 RESISTANCE TO, AND BIOSYNTHESIS OF, THE POLYETHER-IONOPHORE ANTIBIOTIC TETRONASIN IN S. longisporoflavus, KENNETH J. LINTON, IAIN S. HUNTER, INSTITUTE OF GENETICS, UNIVERSITY OF GLASGOW, CHURCH STREET, GLASGOW, G11 8JS.
Two non-identical sequences (tetR1 and tetR5) were isolated from S. longisporoflavus and found to increase the tolerance of S. lividans to the antibiotic tetronasin. tetR5 also conferred resistance upon a tetronasin-super-sensitive strain of S. albus to a level comparable with that found in wild type S. longisporoflavus and may represent the first example of the isolation of an ionophore resistance determinant.

Cross hybridization identified a 5kb BamHI fragment from total DNA of S. longisporoflavus with homology to actI. Paradoxically a 4.3kb BamHI fragment from S. cinnamonensis with good sequence homology to actI (J. A. Robinson, personal communication) hybridized with a 7kb fragment from S. longisporoflavus. Both fragments were cloned and interestingly the 7kb fragment (MB74) was contiguous with sequences which efficiently hybridized to tetR1.

CC 314 CHARACTERIZATION AND CLONING OF STREPTOMYCES AUREOFACIENS RESISTANCE DETERMINANTS, Lomovskaya N.D.,Sezonov G.V., Chinenova T.A., Isaeva L.M., Voeykova T.A. Institute of Genetics and Selection of Industrial Microorganisms, Moscow, USSR. The study of determinants of resistance in S.aureofaciens S799, the producer of chlortetracycline (Ctc) allowed to identify inducible mtr gene(s) which may simultaneously provide increase in strain resistance to autogenous antibiotic Ctc and a number of macrolides in the presence of exogenous inductors in the medium, such as both macrolides and Ctc. Possible adaptive functions of mtr gene(s) are being discussed. Mutants are isolated ensuring constitutive level of resistance to Ctc and macrolides. A number of mutants have increased Ctc biosynthesis. Cloning of mtr gene(s) may reveal the extent of distribution of mtr-like genes in Streptomyces strains. Chromosomal localization of genes for Ctc and oxytetracycline (Otc) biosynthesis in S.rimosus on DNA fragments of equal size is inferred according to data on their hybridization with actI and actIII genes controlling synthesis of polyketides. Also, differences between DNA restriction maps of Ctc and Otc gene clusters were observed. Fragments containing ctrA and ctrB resistance genes linked to Ctc biosynthesis genes in S.aureofaciens were cloned, which demonstrated similarity and differences between restriction maps of DNA fragments with resistance genes of S.aureofaciens and S.rimosus.

CC 315 ANTIBIOTIC PRODUCTION IN STREPTOMYCES COELICOLOR: DETECTION AND ASSAY BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, J. Clark Mason, Glynn Hobbs, Anthony I.C. Obany & Stephen G Oliver, Biotechnology Centre & Department of Biochemistry & Applied Molecular Biology, UMIST, Manchester, M60 1QD, U.K. Using the model organism Streptomyces coelicolor A3(2) we have examined the antibiotic production in continuous and batch cultures. We shall report on the development of convenient assays for the antibiotics, methylenomycin, actinorhodin and undecylprodigiosin, using reverse phase high performance liquid chromatography under isocratic conditions, and their identification by the spectral characteristics of the compounds. The methodology has enabled not only quantitative measurement of the metabolites but also the identification of possible derivatives of the antibiotics arising in culture supernatants. For example, it has been possible to follow the production of methyleneomycin and its derivative desepoxy methyleneomycin during continuous and batch cultures. Progress on the identification of other antibiotic derivatives arising during the organism's growth is reported.

CC 316 CHARACTERIZATION OF THE PUTATIVE TETRACENOMYCIN POLYKETIDE CYCLASE GENE, *tcm* VI, FROM STREPTOMYCES GLAUCESCENS: NUCLEOTIDE SEQUENCING AND PROMOTER ANALYSIS, Haideh Motamedi, Randi L. Rubin, Stanley L. Streicher, H. Nakayama[§] and C. Richard Hutchinson[§], Department of Infectious Disease Research, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065; [§]School of Pharmacy and Department of Bacteriology, University of Wisconsin, Madison, WI 53706.
Streptomyces glaucescens tetracenomycin VI (*tcm* VI) mutants accumulate an unstable tricyclic compound, Tcm F1, instead of the tetracyclic Tcm F, indicating that these mutants are defective in a cyclization step of the Tcm C pathway. The *tcm* VI mutation maps next to polyketide synthase genes (*tcm* lade) which together form the early gene subcluster of the Tcm C biosynthetic genes. Sequencing and analysis of a 1.6 kb *Bgl* II- *Bam*HI DNA fragment containing *tcm* VI using the UWGCG FRAME and CODON PREFERENCE programs, revealed the presence of a complete open reading frame (*tcmVI orf*) that ends one nucleotide before the beginning of the *tcmla orf1*. The *tcm*VI orf thus is possibly translationally coupled to *tcmla orf1*, which in turn is coupled to *tcmla orf2*. *Tcmla orf1* and *orf2* are believed to encode, respectively, the 45 kD and 42 kD subunits of the β-ketoacyl synthase that catalyzes the formation of the decaketide intermediate leading to Tcm F1.

Using the promoter probe vectors described by Ward et. al. (1986) we have found two strong promoters within the sequenced fragment that are divergently transcribed. One of these promoters is located in front of *tcmVI* and thus is likely to be responsible for expression of a polycistronic transcript containing at least three orfs. Possible transcriptional start site(s) and other regulatory elements within *tcm* VI region will be discussed.

CC 317 BIOSYNTHESIS OF A MODIFIED POLYKETIDE CONTAINING TWO AMINO ACID-DERIVED RESIDUES, Jennifer B.K.Nielsen, Merck and Co.,P.O. Box 2000, Rahway, NJ 07065.
Labelling studies to establish the biosynthesis of efrotomycin, a disaccharide of aurodox, have addressed its polyketide nature, demonstrating the incorporation of 13 acetates, and one each of propionate and butyrate into its backbone. Left unresolved was the origin of two nitrogen residues, one in a methylated peptide bond in the terminal pyridone ring and one in a peptide bond with the butyrate carbonyl group. The pyridone nitrogen is derived from β-alanine (see abstract by G.Darland and L.Kaplan) and the other from glycine, which is incorporated intact into C23,C24, N25, with high enrichment. The mode of incorporation resembles that of non-ribosomal synthesis of peptide antibiotics. Activating enzymes for β-alanine and glycine have been purified by ammonium sulfate precipitation and several ion-exchange steps, by following amino acid dependent pyrophospate-ATP exchange reactions and ATP dependent binding of the amino acids into thioester bonds with protein. Thioester linkage is demonstrated by release of the amino acid by performic acid, and not by formic acid. The β-alanine activating enzyme is a monomer of 120kD which does not catalyse N-methylation . The methylation, using S-adenosyl methionine as donor, occurs only after covalent attachment of β-alanine to protein. Further work is being done to characterize the glycine activating enzyme, and the enzymes catalyzing β-alanine methylation and peptide bond formation.

CC 318 RESISTANCE TO SPIRAMYCIN IN *Streptomyces ambofaciens*,THE PRODUCING ORGANISM. Jean-Luc PERNODET, URA CNRS D1354, Université Paris-Sud, F-91405 Orsay Cedex, France.

Streptomyces ambofaciens produces spiramycin, a macrolide antibiotic which inhibits protein synthesis by a mechanism involving the binding of the antibiotic to the large subunit of the ribosome. In *S. ambofaciens* the level of resistance towards spiramycin varies with the age of the culture. A young mycelium, not yet producing, is sensitive to spiramycin. But an old, producing mycelium is highly resistant to spiramycin. We have shown that *S. ambofaciens* possesses a spiramycin inducible ribosomal resistance to spiramycin. Studies with spiramycin non-producing mutants suggest the existence in *S. ambofaciens* of other resistance mechanism(s). A genomic library of *S. ambofaciens* DNA was constructed and introduced in *Streptomyces griseofuscus*, a strain sensitive to spiramycin. This allowed us to isolate spiramycin resistance determinants from *S. ambofaciens*. Further characterization was carried out for one of these cloned determinants which confers spiramycin inducible ribosomal resistance to macrolides and lincosamides.

CC 319 ISOLATION AND SEQUENCE ANALYSIS OF AN actI/III HOMOLOGOUS GENOMIC REGION FROM Kibdelosporangium aridum, PIECQ M., DEHOTTAY Ph., MORRISSEY J., BIOT A., DUSART J.

Ardacin is a new glycopeptide antibiotic naturally produced by the Actinomycete Kibdelosporangium aridum.
Its biosynthetic pathway involves the condensation of several acetates probably via a polyketide synthetase (PKS).
The K.aridum genomic DNA was found to hybridize with actI, the first PKS gene isolated from Streptomyces (Malpartida F. & Hopwood D.A. (1984) Nature, 309, 462).
The actI fragment was used as a probe to identify the homologous BamHI fragment from K.aridum in a shot-gun cloning experiment via the pBR322 vector.
The 2.8kb isolated fragment, called ardI, was sequenced and compared to other PKS gene sequences. It was also used as a probe to isolate large K.aridum genomic adjacent regions from a shuttle cosmid library. 3 hybridizing cosmids cover together a 50kb genomic region; a restriction map was drawn. This region contains also an actIII (another S.coelicolor PKS subunit gene) homologous fragment, 15kb from the actI homologous fragment.
The cosmids were introduced in S.lividans and checked for antibiotic production : at this time, no reproducible production has been detected, the cosmids being very unstable in that host. Other expression tests are currently performed using other Actinomycete hosts.

CC 320 MOLECULAR CLONING STUDIES OF BLEOMYCIN BIOSYNTHESIS.
Francis J. Schmidt, Michael Calcutt, and Linnea Ista. Department of Biochemistry, University of Missouri-Columbia, Columbia MO 65212.
The antitumor glycopeptide antibiotic bleomycin is produced by a Streptoverticillum (S. verticillus) species. We report the isolation of cosmid clones encoding bleomycin biosynthetic functions by the selection of antibiotic resistance expression in recipient strains of E. coli. These clones express antibiotic biosynthetic functions linked to the resistance marker as evidenced by the production of a polymeric pigment derived from amino acids in E. coli. A genetic transfer system from S. lividans to Streptoverticillum has been identified.

CC 321 ANALYSIS AND SEQUENCE DETERMINATION OF carA, A CARBOMYCIN RESISTANCE GENE FROM STREPTOMYCES THERMOTOLERANS, Brigitte Schoner and Stanley Burgett, Molecular Genetics Research, Lilly Research Laboratories, Indianapolis, IN 46285
A plasmid (pOJ158) conferring carbomycin resistance was isolated from Streptomyces griseofuscus transformed with a S. thermotolerans DNA library prepared in pIJ702. The carbomycin resistance gene (carA) was localized by subcloning to a 4 kb fragment whose DNA sequence was determined. This sequence contains two adjacent open reading frames (ORF), one of which was shown to encode a 60 kD protein in an in vitro transcription/translation system. The region containing this ORF hybridizes to a tylosin resistance gene (tlrC) from Streptomyces fradiae and to a spiramycin resistance gene (srmB) from Streptomyces ambofaciens.

CC 322 IDENTIFICATION OF TWO POSSIBLE REGULATORY LOCI FOR DAUNORUBICIN BIOSYNTHESIS IN *STREPTOMYCES PEUCETIUS*, Kim J. Stutzman-Engwall, Sharee L. Otten and C. Richard Hutchinson, School of Pharmacy, Univ. of Wisconsin-Madison, Madison, WI 53706. We are investigating the organization and function of the daunorubicin production genes from *S. peucetius* ATCC 29050. We have found four distinct, non-overlapping regions of DNA that are involved in antibiotic production or resistance and have studied the Group IV region in detail. This region contains most if not all of the genes for daunorubicin biosynthesis. Cosmid clones from Group IV, when transformed into *S. peucetius* 29050 and 27952 and *S. peucetius* blocked mutants, can cause up to a 100-fold increase in the production of ε-rhodomycinone (a daunorubicin pathway intermediate) and up to an 8-fold increase in daunorubicin production. By subcloning DNA from this region we were able to identify 2 fragments that appear to govern the function of other genes in the cluster and cause the enhanced production of metabolites, specifically ε-rhodomycinone and daunorubicin. A 2.0 kb *Bam*HI/*Bgl*I fragment stimulates ε-rhodomycinone production in *S. peucetius* and actinorhodin production in *S. lividans*. A 1.9 kb *Bam*HI fragment stimulates both ε-rhodomycinone and daunorubicin production in *S. peucetius*. Both fragments restore ε-rhodomycinone and daunorubicin production when transformed into a putative *S. peucetius* regulatory mutant. These results suggest that *S. peucetius* may have multiple 'regulatory genes', examples of which have not yet been found in other antibiotic biosynthetic gene clusters of *Streptomyces*. These DNA fragments are currently being characterized both structurally and functionally.

CC 323 PLASMID EFFECTS ON SECONDARY METABOLITE PRODUCTION BY *STREPTOMYCES THERMOARCHAENSIS*. D. Ian Thomas, Jonathan H. Cove, [1]Simon Baumberg, [2]Carol A. Jones and [2]Brian A.M. Rudd, Departments of Microbiology and [1]Genetics, University of Leeds, Leeds, LS2.9JT and [2]Glaxo Group Research Ltd, Greenford Road, Greenford, UB6.0HB, UK.
The thermophilic streptomycete, *Streptomyces thermoarchaensis* has been shown to produce polyketide secondary metabolites with antihelminthic activity. These products are structurally similar to the avermectins and have been termed archaemycins. *S. thermoarchaensis* also produces 21-hydroxyoligomycin A which is chemically distinct from the archaemycins. This report describes the effects on secondary metabolite production of transforming *S.thermoarchaensis* with plasmid DNA. Plasmids of the pIJ101 series eg. pIJ702, and of SCP2* eg. pIJ922 were found to depress selectively the production of archaemycins but not oligomycin. It was confirmed that this effect was specifically plasmid mediated, since cured strains demonstrated normal production profiles. Subsequent retransformation by plasmid DNA of previously cured strains again depressed archaemycin production. All plasmids tested were highly unstable and it was necessary to include an appropriate antibiotic (usually thiostrepton) in the growth medium. However, it is unlikely that thiostrepton was responsible for the effect, since (a) production of archaemycins was depressed in antibiotic free media when the inoculum was grown in the presence of thiostrepton, and (b) the non-plasmid containing strain produced both archaemycins and oligomycin in the presence of sub MIC levels of thiostrepton. The effect was not mediated by the thiostrepton resistance gene since plasmids carrying alternative selectable markers (neomycin, pIJ58, and viomycin, pIJ355) instead of thiostrepton resistance also selectively depressed archaemycin but not oligomycin production. At present, there is no evidence that *S.thermoarchaensis* carries indigenous plasmids. Current effort is directed towards elucidating the mechanism of this selective, plasmid-mediated depressive effect on secondary metabolism.

CC 324 Cloning of grm, a gentamcin resistance gene from Micromonospora purpurea
Branka Vasiljevic and Eric Cundliffe, Department of Biochemistry and Leicester Biocentre, University of Leicester, Leicester LE1 7RH, UK.

A second gentamicin resistance determinant (grm) has been cloned from Micromonospora purpurea an actinomycete that produces gentamicin. Like the first one (kgm) this gene confers resistance at the level of the ribosome to aminoglycoside antibiotics of the kanamycin and genetamicin families. Again, like the kgm gene, grm encodes a methylase enzyme which acts upon 16S rRNA and generates a single residue of 7-methylguanosine. The site of methylation due to the kgm product was previously shown to be residue G-1405. The grm product acts at a different site within 16S rRNA.

CC 325 FUNCTIONS OF PUTATIVE POLYKETIDE SYNTHASE GENES FROM MIDECAMYCIN AND SPIRAMYCIN PRODUCING STRAINS IN *STREPTOMYCES COELICOLOR ACTI* AND *ACTIII* MUTANTS, Wang Yiguang, Tang Li, Zhu Xuewei, Xiu Xiaoming, Zeng Ying & C. R. Hutchinson†, Institute of Medicinal Biotechnology, CAMS, Beijing, China; †School of Pharmacy and Department of Bacteriology, University of Wisconsin, Madison, WI

The finding of highly conserved regions in the sequences of the products believed to be encoded by polyketide synthase genes in several *Streptomyces* spp. suggests that some of the individual components of polyketide synthases from one species of these bacteria could function in the biosynthesis of polyketide antibiotics by another species. To test this idea, genomic libraries of DNA from the midecamycin-producing *Streptomyces mycarofaciens* 1748 and spiramycin-producing *Streptomyces* sp. 1941 were constructed in the multicopy *Escherichia coli-Streptomyces* shuttle cosmid vectors pNJ1 (obtained from Abbott Laboratories) and pMNJ1 (a derivative of pNJ1 constructed our laboratory), respectively. The following plasmids containing putative polyketide synthase genes that hybridized to the *Streptomyces coelicolor actI* or *actIII* genes were isolated from these libraries and subcloned in the multicopy *E. coli-Streptomyces* shuttle vector pWHM3 (obtained from C. R. Hutchinson): pCG2 (a 2.42 kb DNA segment from *S. mycarofaciens* that hybridized to *actI*), pCG4 (a 4.0 kb DNA segment from *Streptomyces* sp. 1941 that hybridized to *actI*), and pCB4 (a 4.02 kb DNA segment from *S. mycarofaciens* that hybridized to *actIII*). Transformation of the *S. coelicolor* TK17 (*actI*) blocked mutant with pCG2 and pCG4 and the *S. coelicolor* TK18 (*actIII*) blocked mutant with pCB4 resulted in the production of antibiotic substances different from midecamycin, spiramycin and actinorhodin according to the results of TLC and bioassay analyses. Transformation of *Streptomyces ambofaciens* with pCG2 and pCG4 resulted in a reproducible 4 to 6-fold overproduction of spiramycin in liquid cultures.

CC 326 ORGANIZATION OF A CLUSTER OF ERYTHROMYCIN GENES IN *SACCHAROPOLYSPORA ERYTHRAEA*, **J. Mark Weber, Judith O. Leung, Gregory T. Maine, Rica Potenz, Janet P. DeWitt, Bioprocess Development, Abbott Laboratories, North Chicago, IL 60064**

We used a series of gene disruptions and gene replacements to mutagenically characterize 30 kb of DNA in the erythromycin resistance gene (*ermE*) region of the *Saccharopolyspora erythraea* chromosome. Four new loci involved in the biosynthesis of erythromycin were found, *eryBI, eryCI, eryCII* and *eryH*; and three known loci, *eryAI*, *eryG* and *ermE* were further characterized. A new ery phenotype, EryH, was marked by a) the accumulation of the precursor 6-deoxyerythronolide B, suggesting a defect in the operation of the C-6 hydroxylase system and b) a block in the synthesis or attachment reactions for the first sugar group. Analyses of *ermE* mutants indicated that *ermE* is the only gene required for resistance to erythromycin, and that it is not necessary for production of erythromycin. Mutations in the *eryB* and *eryC* loci were similar to previously reported chemically induced *eryB* and *eryC* mutations blocking synthesis or attachment of the two erythromycin sugar groups. Insertion mutations in *eryAI*, the macrolactone synthetase, defined the largest (at 9kb) transcription unit of the cluster. These mutants help to define the physical organization of the erythromycin gene cluster, and the *eryH* mutants provide a source for the erythromycin precursor 6-deoxyerythronolide B.

CC 327 CLONING OF A FOURTH RESISTANCE GENE FROM THE TYLOSIN PRODUCER, *Streptomyces fradiae*. Magdalena Zalacain and Eric Cundliffe, Department of Biochemistry, University of Leicester, Leicester LE1 7RH. ENGLAND.

A fourth gene, *tlrD*, conferring resistance to some MLS antibiotics has been isolated from *Streptomyces fradiae* and cloned in *Streptomyces lividans*. This determinant encodes an enzyme which introduces a single methyl group into 23S ribosomal RNA. The nucleotide (A-2058) that is monomethylated constitutively by this enzyme is also the target for the *tlrA* protein, that is produced inducibly in the same strain grown in tylosin production medium. Under the latter conditions, A-2058 is dimethylated. Southern analysis revealed that *tlrD* is not closely similar to other MLS resistance determinants from actinomycetes.

Gene Transfer, DNA Amplifications, Genome Structure and Expression Systems

CC 400 MOLECULAR CHARACTERIZATION OF STREPTOMYCES VENEZUELAE PHAGE VWB, Jozef Anné, Lieve Van Mellaert & Hendrik Eyssen. Rega Institute, Laboratory of Microbiology, K.U.Leuven, B-3000 Leuven, Belgium.

The temperate bacteriophage VWB (47.3 kb) can infect Streptomyces venezuelae ETH14630 (= ATCC40755) and Streptomyces exfoliatus ATCC12672. It is not infectious for any of 5 other S.venezuelae strains tested. However, VWB phage DNA can be introduced in these non-infectable strains by transfection. Phage DNA was then propagated and it was able to lysogenize the host. This was clearly shown with VWB04, a VWB phage derivative containing a thiostrepton resistance gene. S.venezuelae strains lysogenized by VWB04 became thiostrepton resistant, and VWB04 phage particles were released from the transfected S.venezuelae strains. By comparison of restriction fragments of VWB DNA, either free or integrated in the chromosome of the lysogen, the attachment site was localized.

Polyacrylamide gel electrophoresis of the phage proteins revealed at least 17 different protein bands with 3 major proteins estimated 16.5, 27.2 and 43 kilodalton in size. The N-terminal amino acid sequence of these supposed major head and tail proteins was determined. Localization of the corresponding DNA sequences on the phage genome using oligonucleotides synthetized on the basis of the N-terminal amino acid sequences indicated that the genes coding for the major structural proteins are clustered, as has been observed for other bacteriophages.

CC 401 ABILITY OF STREPTOMYCES SPECIES TO COLONIZE ARTIFICIAL SOIL AGGREGATES AND EXCHANGE A CONJUGATIVE PLASMID DURING COLONIZATION, Bruce H. Bleakley and Don L. Crawford, Bacteriology and Biochemistry Department, University of Idaho, Moscow ID 83843. Spores of a Streptomyces lividans strain (donor) bearing the recombinant conjugative plasmid pIJ303, which codes for thiostrepton resistance, and spores of a plasmid-free Streptomyces parvulus strain (recipient) were added together to finely-sieved, sterile silt loam soil. This bulk soil was adjusted to either approximately 20%, 35% or 45% of the soil's water holding capacity. To this inoculated bulk soil were added sterile, nutrient-amended, artificial soil aggregates. After incubation to allow streptomycete growth and aggregate colonization, both the sieved bulk soil and the aggregates were assayed for numbers of transconjugants by spread-plating on thiostrepton-agar selective medium. This allowed estimation of parental ability to colonize nutrient-rich soil sites at different soil moisture levels, and comparison of plasmid exchange frequency in the nonamended bulk soil versus that at the nutrient-rich soil aggregates. The effects of different clay minerals on substrate colonization and plasmid exchange were also evaluated with this experimental system.

CC 402 IS466-MEDIATED TRANSPOSON MUTAGENESIS IN STREPTOMYCES GENERATES AUXOTROPHIC MUTANTS, Rosanna Di Guglielmo, Claus Conzelmann, Fiona Flett and John Cullum[+], Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester M60 1QD, U.K.; [+]Lehrbereich Genetik, Universitat Kaiserslautern, FRG

We constructed a transposon-delivery vector (pMT2077) using two copies of the insertion sequence IS466. This element was isolated from S.coelicolor A3(2) together with the agarase gene (Kendall & Cullum, 1986). It is also present on the giant linear plasmid SCP1 and is believed to mediate the integration of SCP1 into the S.coelicolor chromosome. The suicide vector pMT2077 consists of a viomycin resistance gene (vph) flanked by two inverted copies of IS466. This artificial composite transposon is cloned into a derivative of the temperature-sensitive plasmid pMT660 (carrying thiostrepton resistance). After transformation of S.lividans TK64 the colonies were grown at 39°C without antibiotic to select against the vector. C. 4000 colonies resistant to viomycin but sensitive to thiostrepton were screened for auxotrophic mutants. We found 23 auxotrophs (i.e. 0.6%) mainly requiring aromatic amino acids; they can be divided into six groups according to the substances required: 1) phenylalanine 2) tryptophan 3) phenylalanine + tyrosine 4) phenylalanine + tyrosine + tryptophan 5) arginine 6) others.

CC 403 HOMOLOGY TO *Streptomyces avermitilis* REITERATED SEQUENCES FOUND IN A VARIETY OF STREPTOMYCETES. F. Foor, K. Gewain, and D. MacNeil. Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

Streptomyces avermitilis produces avermectin, a complex of eight structurally related polyketide anthelmintic agents. The cloning and analysis of the avermectin biosynthetic gene cluster from *S. avermitilis* has led to the discovery of two groups of reiterated or related sequences (rsDNA) in this region. Reiterated sequences are commonly found in *Streptomyces*. In particular, rsDNA elements are present in the *S. fradiae* biosynthetic gene cluster for tylosin, a polyketide antibiotic [Baltz and Seno, *Ann. Rev. Microbiol.* 42 (1988) 547-574]. We have used DNA probes derived from the two *S. avermitilis* rsDNA groups to test for the presence of similar sequences in a variety of *Streptomyces* under low stringency conditions. Sequences homologous to both rsDNA groups were found in multiple copies in a number of species. Low homology was seen in DNA isolated from *S. fradiae*. Two of the three strains with rsDNA having the highest homology produce nemadectin, a polyketide structurally related to the avermectins. The third strain produces the polyketide L-155,175.

CC 404 CHARACTERIZATION OF THE STREPTOMYCETE BACTERIOPHAGES FP43 AND FP22, Donald R. Hahn, Margaret A. McHenney, and Richard H. Baltz, Molecular Genetics Research, Lilly Research Laboratories, Indianapolis, IN 46285

We have characterized two broad host range bacteriophages with properties which may be useful for gene transfer in Streptomyces. The temperate phage, FP43, has a genome of 47 kb which has 65% G+C content. The genome is circularly permuted and terminally redundant, suggesting a headful packaging mechanism. The phage genome has been restriction mapped and several phage functions have been mapped. A plasmid containing the phage pac site can be transduced by FP43 into approximately 80% of all streptomycete strains tested. FP22 has a very broad host range and a rather unique genome composition. Unlike most other streptomycete phages, FP22 DNA has less than 50% G+C content and contains no sites for most of the known Streptomyces restriction systems. The FP22 genome is 130 kb in size and packaged by a cos type mechanism. NaCl and pyrophosphate resistant deletion mutants have been isolated and mapped. Analysis of the deleted region indicates that at least 15 kb of FP22 DNA is dispensable.

CC 405 ISOLATION AND CHARACTERIZATION OF HERBICIDE METABOLIZING CYTOCHROME P450SU1 DELETION MUTANTS OF STREPTOMYCES GRISEOLUS , Patricia A. Harder, James A. Romesser, Charles A. Omer, and Daniel P. O'Keefe. Central Research & Development Dept., E.I. DuPont, Experimental Station, Wilmington, DE 19800-0228 Metabolism of sulfonylurea herbicides by the soil bacterium *Streptomyces griseolus* ATCC11796 has been shown to be carried out via two herbicide inducible cytochrome P450 mono-oxygenases, P450SU1 and P450SU2. The genes for these P450 proteins and their corresponding iron sulfur proteins are encoded on two separate operons, *suaB,C* and *subB,C*, respectively. Mutants of *S. griseolus* isolated following treatment with nitrosoguanidine, UV, or regeneration of protoplasts were found to contain deletions of the *suaB,C* genes. Metabolism of sulfonylurea herbicides by these strains is due solely to the expression of the *subB,C* genes. While *S. griseolus* was shown to contain a 450 kb linear plasmid by PFGE, the *suaB,C* and *subB,C* genes were found to be located on the chromosome. The size of the deletion in the mutants has not been determined , but includes at least 15 kb of DNA. The *sua B,C* genes were reintroduced into mutant strains via transformation and recombination. Mutant strains transformed with pIJ922 containing a 2.4 kb *Bam*H1 fragment which includes the *sua B,C* genes now show constituitive expression of the genes for P450SU1, indicating the absence of regulatory genes in the deletion mutants. Mutant strains which have reacquired the *sua B,C* genes via recombination show regulated expression of the genes for P450SU1.

CC 406 ORGANIZATION OF THE act REGION OF Streptomyces coelicolor, David A. Hopwood[1], Jose Caballero[1], Miguel Fernandez[2], Eduardo Martinez[2] and Francisco Malpartida[2], [1]John Innes Institute, Norwich, U.K. and [2]Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Spain.
Molecular genetic analysis of the ca. 22 kb region of the S. coelicolor chromosome that carries the complete set of biosynthetic genes (act) for the isochromanequinone antibiotic, actinorhodin, led to the location of genes controlling steps in the biosynthesis of the polyketide-derived carbon chain of the antibiotic, genes for intermediate and late enzymes of the biosynthetic pathway, as well as a central regulatory region in the gene cluster. DNA sequencing is now revealing a series of open reading frames that correspond to biosynthetic, resistance and regulatory genes. The current picture developed from these studies will be described.

CC 407 CHLORAMPHENICOL-SENSITIVE STRAINS OF STREPTOMYCES LIVIDANS SUPPORT CHROMOSOMAL DNA REITERATION MEDIATED BY THE AMPLIFIABLE UNIT OF DNA OF STREPTOMYCES ACHROMOGENES SUBSP. RUBRADIRIS, AUD-Sar 1. Ulfert Hornemann, Xiao Yu Zhang, and Tony A. Klink, School of Pharmacy, University of Wisconsin, Madison, WI 53706
We previously reported highly efficient transferable 8.0 kb DNA amplification mediated by the 8.8 kb AUD-Sar 1 carried on the temperature sensitive replicon pMT660 in S. lividans strain CO117. Subsequent experiments with the same vector in the wild-type S. lividans strain 1326, which were repeated several times, failed to reveal the occurrance of the amplification phenomenon. During the previous experiments the formation of a 5.7 kb reiterated sequence was also observed in a minority of the isolates. Since the latter amplification is normally seen only in chloramphenicol-sensitive strains of S. lividans, strain CO117 was examined and found to be chloramphenicol sensitive. Strain CO117 is also auxotrophic but its requirements appear to be complex. Experiments with the chloramphenicol-sensitive S. lividans strain M252 indicate that AUD-Sar 1-mediated 8.0 kb DNA amplification occurs reproducibly, albeit at lower frequency than in strain CO117. Sometimes we observed the occurrance of both the 8.0 kb and the 5.7 kb amplified DNA sequences in certain isolates. Both amplified sequences were shown to be present in three separate colonies obtained by streaking a doubly amplified strain. As reported by other laboratories chloramphenicol-sensitive S. lividans strains carry a large deletion which may be a prerequisite for DNA amplification, but the nature of this requirement awaits to be illuminated.

CC 408 A RECOMBINANT SYSTEM FOR EXTRACELLULAR PRODUCTION OF POLYPEPTIDES BASED ON A STARCH-INDUCIBLE α-AMYLASE PROMOTER OF *STREPTOMYCES*,
Bengtåke Jaurin, Micael Granström and Annika Osterman, Department of Cell and Microbiology, National Defence Research Establishment, S-901 82 Umeå, Sweden. A *Streptomyces* strain, isolated from a compost, was found to express high levels of extracellular α-amylase. A gene bank of this strain was constructed in *Streptomyces lividans*. One clone was identified that expressed high levels of α-amylase in an extracellular form. The synthesis of α-amylase in *S. lividans* was induced by maltose or starch, but repressed with glucose. The carbon source dependent α-amylase expression was found to be regulated at the level of transcriptional initiation. Primer extension analysis revealed the start point of transcription. The location and structure of the α-amylase promoter region as well as the signal sequence of the extracellular enzyme were established.

This α-amylase gene provides the basis for a recombinant system for starch-inducible polypeptide production in *Streptomyces*. DNA containing the inducible promoter as well as the signal sequence was used to construct an expression vector, to which coding sequences for polypeptides were fused. Results on inducible and extracellular expression in *S. lividans* of eucaryotic proteins (acetylcholinesterase, α-interferon and superoxide dismutase) will be discussed.

CC 409 CHARACTERIZATION OF THE GENETIC ELEMENTS INVOLVED IN SITE-SPECIFIC INTEGRATION OF PLASMIDS pSE211 AND pSE101 FROM Saccharopolyspora erythraea, Leonard Katz, David Brown and Kenneth Idler, Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60064
Several actinomycete plasmids integrate site-specifically into one or more actinomycete hosts. Plasmids pSE211 and pSE101 integrate site-specifically by a non-replicative, conservative process in Saccharopolyspora erythraea, analagous to integration of bacteriophages. They replicate autonomously, at moderately high copy number, in Streptomyces lividans. pSE101 can also integrate at multiple sites in S. lividans. Segments of each plasmid have been identified that, when cloned in an E. coli vector carrying the thiostrepton resistance gene, can direct integration of the resulting plasmid at its cognate attB site in Sac. erythraea. The segment from pSE211 consists of an attP site and two adjacent open reading frames which are read in the same direction. Orf1 encodes a 98 amino acid basic polypeptide that contains a helix-turn helix domain and shares homology with Xis proteins of bacteriophages. Orf2 encodes a 438 amino acid polypeptide, termed Int, that shares C-terminal homology with the "Integrase" family of site-specific recombinases. Both Orf1 and Int are required for stable integration at attB. The segment of pSE101 involved in integration shows a similar organization. The attP sites of the two plasmids do not share significant sequence homology, but substantial homology at both the DNA and protein level of the two integrases was observed. The attB sites of each plasmid appears to correspond to a structural gene for t-RNA: Phe-tRNA for pSE211; Thr-tRNA for pSE101. The C-terminal segment of each tRNA gene is present at attP of each plasmid so that integration does not cause disruption of the structural gene. The sequences of both attP and attB of pSE211 are identical to the corresponding sequences of the plasmid pMEA100 from Nocardia mediterranei.

CC 410 TRANSFERABLE AUD-SAR 1-DEPENDENT DNA AMPLIFICATION AS STUDIED WITH AUD DELETIONS AND AN INSERTION. Tony A. Klink, Ulfert Hornemann, and Xiao Yu Zhang, School of Pharmacy, University of Wisconsin, Madison, WI 53706.
pBV730ts, which is composed of AUD-Sar 1, the 8.8 kb amplifiable unit of DNA of Streptomyces achromogenes subsp. rubradiris, and the temperature sensitive replicon pMT660, can mediate chromosomal 8.0 kb DNA reiteration in chloramphenicol-sensitive strains of Streptomyces lividans under appropriate growth conditions. The Pvu II digest of the reiterated DNA shows 1.0, 2.5, and 4.5 kb bands; the middle band constitutes the connecting band between repeated units. AUD-Sar 1 carries three Pvu II sites at position 0.7, 1.7, and 6.2 and a unique Sca I site at position 4.5. The first Pvu II site is located near the left of two AUD 0.8 kb direct repeats, whereas the third Pvu II site is located near a spectinomycin resistance gene carried by the AUD. A pBV730ts derivative which carries an AUD lacking the 1.0 kb Pvu II fragment afforded a 7.0 kb highly reiterated DNA sequence which upon digestion yielded bright 2.5 and 4.5 kb Pvu II bands. Thus the AUD sequences between position 0.7 and 1.7 do not appear to be required for amplification. In contrast pBV730ts derivatives either lacking the left direct repeat or the two AUD internal Pvu II bands failed to yield amplified DNA sequences, suggesting that the left direct repeat and the 4.5 kb Pvu II fragment contain sequences required for amplification. pBV738, a pBV730 derivative carrying the 1.2 kb neomycin resistance gene npt in the Sca I site afforded a 9.2 kb highly reiterated sequence which upon digestion yielded 1.0, 2.5, and 5.7 kb Pvu II bands demonstrating co-amplification of the npt gene.

CC 411 CLONING AND EXPRESSION OF CHOLESTEROL OXIDASE FROM RHODOCOCCUS RHODOCHROUS IN STREPTOMYCES LIVIDANS, Susan Long, Joseph A. Videler and Gary R.Ostroff, Genzyme Corporation, 75 Kneeland Street, Boston, MA 02111.

Cholesterol oxidase (E.C. 1.1.3.6) catalyses the oxidation of cholesterol to cholestenone. Commercial preparations of the enzyme, obtained from a number of microbial sources, are widely used in clinical assays to determine serum cholesterol levels.

We have cloned the cholesterol oxidase gene from R. rhodochrous in S. lividans. Recombinant clones were identified by screening a genomic bank of R. rhodochrous DNA using a simple plate assay. Recombinant cholesterol oxidase production in liquid media was investigated and it was found that the S. lividans clones constitutively expressed cholesterol oxidase as a secreted enzyme. This was in contrast to the native cholesterol oxidase which is produced as an intrinsic membrane-bound protein and requires the presence of an inducer. The purified cholesterol oxidase from S lividans and R. rhodochrous did however have similar enzyme activities suggesting that the recombinant enzyme could be used in diagnostic cholesterol assays.

CC 412 SECRETION OF GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF) IN STREPTOMYCES LIVIDANS, Lawrence Malek, Gisela Soostmeyer, Cheryl Davey, Phyllis Krygsman, Jean Compton, Jane Gray, Teresa Zimny and Donald Stewart. Cangene Corporation, Mississauga, Ontario, Canada L4V 1T4. An expression vector for heterologous protein secretion was constructed from Streptomyces plasmid pIJ680 by replacement of the promoter and coding region of the aminoglycoside phosphotransferase (aph) gene of Streptomyces fradiae with a subcloned aph promoter joined to a sequence encoding the Streptomyces griseus protease B signal peptide. A gene encoding human GM-CSF was designed for Streptomyces codon usage and constructed from oligonucleotides. The synthetic GM-CSF gene was inserted into the expression vector and the resulting construction was used to transform S. lividans. GM-CSF was secreted into the medium at levels of approximately 10 to 15 mg/l. The secreted GM-CSF was correctly processed from the signal peptide and was biologically active by in vitro assays. Secretion of GM-CSF could also be directed by hybrid signal peptides derived from Streptomyces plicatus endoglycosidase H, Escherichia coli outer membrane protein A and Bacillus amyloliquefaciens subtilisin. The promoter from the Streptomyces coelicolor agarase gene was also effective for the expression of GM-CSF.

CC 413 CONJUGATIVE PROMOTER PROBE AND EXPRESSION VECTORS DERIVED FROM INTEGRATIVE PLASMID pSAM2, Philippe Mazodier*, Tamara Smokvina** and Frederic Boccard**, *Departement des Biotechnologies, Institut Pasteur, 75724 Paris Cedex 15, France, **Laboratoire de Biologie et Genetique Moleculaire, Universite Paris-Sud, F-91405 Orsay Cedex, France

pSAM2 is an 11kb element found integrated in Streptomyces ambofaciens ATCC 23877. A fragment of pSAM2 allowing specific integration at the Streptomycetes attB site was characterised and cloned in pBR329 in Escherichia coli. A conjugative version of this plasmid was constructed by subcloning the origin of transfer oriT of promiscuous plasmid RK2. This allowed direct transfer and insertion of the vector in Streptomyces lividans genome by simple mating with an E. coli donor strain. Two derivatives of this conjugative and integrative vector were constructed and tested: 1) A promoter probe was constructed by insertion of the luxA and luxB genes of the luciferase operon of the luminescent marine bacterium Vibrio harveyi. This permitted measurement of the activity of promoters present at one copy per chromosome by visualizing temporal patterns of light emission. 2) An inducible expression vector was also constructed by insertion of the thiostrepton induced promoter tipA, downstream of a transcription terminator and upstream of a unique BamHI cloning site.

CC 414 EFFECTS OF DIFFERENT PROMOTERS, MEDIA AND SPECIES ON EXPRESSION OF A PLASMID-CLONED, HETEROLOGOUS PARATHION HYDROLASE GENE IN STREPTOMYCETES, B. Pogell, H-L. Zhang, S. Rowland, V. Thaker, and M. Speedie, Dept. of Biomedicinal Chem. & MD Biotech.Instit., Univ. of Maryland, Baltimore, MD 21201.
Levels of secreted parathion hydrolase by S. lividans containing a Flavobacterium opd gene have been shown to fall after reaching a peak early in exponential growth. The effect of a late streptomycete promoter for the sapA spore protein of S. coelicolor (R. Losick et al.) on prolongation of parathion hydrolase production has been studied in S. lividans transformed with various constructs of pIJ702 containing the opd gene. When the sapA promoter was inserted upstream of the opd promoter, lower excreted hydrolase levels were found early in fermentations but higher amounts were excreted after 3-7 days of growth in two different liquid media. Insertion of the sapA promoter into the opd promoter markedly lowered enzyme formation, as did the presence of E. coli pUC18 sequences in the plasmid. The composition of the medium significantly affected enzyme levels. Highest yields were found in fed-batch tryptone-glucose medium with the clone containing both the mel and opd promoters but lacking the sapA promoter (pRYE1). When the opd gene was inserted in reverse orientation and lacked the effect of the mel promoter (pRYE2), enzyme excretion was decreased 20-fold. In addition, S. albus containing pRYE1 produced much lower amounts of extracellular hydrolase than the S. lividans clone.

CC 415 SITE-SPECIFIC INTEGRATING VECTORS FOR STREPTOMYCES, Mark A. Richardson, Stuart Kuhstoss and R. Nagaraja Rao, Molecular Genetics Research, Lilly Research Laboratories, Indianapolis, IN 46285
Plasmid and cosmid vectors that exploit the site-specific recombination systems of ϕC31 and pSAM2 were developed for use in Streptomyces. ϕC31-based vectors transformed Streptomyces protoplasts at a higher frequency than pSAM2-based vectors. Integrates generated by these vectors had high segregational stability. ϕC31-specific functions necessary for integration into the chromosome were localized by deletion analysis. ϕC31 integrating vectors and pIJ101 replicating vectors transformed Streptomyces at high frequencies. A plasmid that included both functions transformed Streptomyces poorly, and a few transformants that grew had altered plasmid DNA structures.

CC 416 EXPRESSION AND SECRETION OF TENDAMISTAT AND TENDAMISTAT - PROINSULIN FUSIONS IN STREPTOMYCES LIVIDANS, GÜnther Rieß, Klaus P. Koller, Klaus Sauber, Eugen Uhlmann, Laslo Vértesy, and Holger Wallmeier, HOECHST AG, 6230 Frankfurt/M. 80, FRG
The gene for the proteinaceous α-amylase inhibitor tendamistat (HOE 467) from Streptomyces tendae was identified on an amplified genomic sequence. After subcloning and sequencing, an open reading frame of 312 bp was found coding for a 74 amino acid mature protein preceded by a 30 amino acid signal peptide. The gene was expressed and secreted from the heterologous host Streptomyces lividans (Koller & Rieß, J. Bacteriol., in press). Using specifically designed oligonucleotide linkers, we have made partial and complete fusions between tendamistat and a monkey proinsulin gene. The fusion proteins were expressed and secreted from S.lividans and easily purified from the culture filtrate. After re-folding and proteolytic digestion with trypsin, biologically active des-Thr(B30) insulin was obtained (Koller et al., Bio/Technology, in press).

CC 417 GENOME PLATICITY ASSOCIATED WITH GENETIC INSTABILITY AND HYPERVARIABILITY IN *STREPTOMYCES AMBOFACIENS*, J.-M. Simonet, B. Decaris, P. Leblond and P. Demuyter, Laboratoire de Génétique et Microbiologie, Université Nancy I, Faculté des Sciences, B.P. 239, 54506 Vandoeuvre, France.

Streptomyces ambofaciens DSM40697 exhibits a high degree of genetic instability revealed by the occurence of about 1 % of pigment-defective colonies from the WT strain. While only 13 % of these mutant colonies show a homogeneous mutant progeny, 87 % give rise to hypervariable progeny.

In the strain DSM40697, the molecular analysis of the total DNA isolated from WT, stable and hypervariable lineages reveals DNA amplifications related to phenotypic hypervariability. Two families of amplifiable unit of DNA, the AUD6 and AUD103 families have been characterized.

In order to detect other rearrangements of the AUD6 region in various mutant strains, hybridization experiments were carried out using the cloned fragment generated by the tandem reiteration of the AUD as a probe. Hybridization patterns reveal no rearrangement in the WT strains, and a high frequency of large deletions overlapping the AUD6 region in the mutant strains isolated not only from the hypervariable lineages, but also from the stable ones. Therefore, deletion events are closely related to the genetic instability. This fact suggest a mechanism of genetic instability through a cascade of mutational events.

A few patterns show one duplication of a sequence homologous to the probe. Similar analysis of subclones of the AUD6 amplified strain reveal a loss of the amplification associated with a rearrangement of the AUD in 30 % of the strains. Thus, the AUD6 region is an unstable region undergoing duplication, amplification or deletion. Hence, this region could be a hot spot for rearrangements involved in the mechanism of genome plasticity.

Pulsed field gel electrophoresis was used in order to detect DNA rearrangements at the level of the whole genome. Analysis of the large restriction fragments generated by the enzymes *AseI* and *DraI* indicates a minimal size for the WT genome of some 6,500 kbp. The restriction patterns of independant stable pigment-defective strains as well as independant hypervariable strains reveal missing bands compared to the WT. Some of the missing bands are common to all the mutants and add up more than 2,000 kbp (i.e., about 30 % of the estimated genome). This was not observed if *HindIII* was used instead of *AseI* or *DraI*. This fact suggests perhaps that the missing bands are not deleted but have undergone structural changes.

CC 418 QUANTITATIVE STUDIES ON PLASMID STABILITY IN *STREPTOMYCES LIVIDANS*. Dawn-Lesley Simpson, Jonathan H. Cove, [1]Simon Baumberg, [2]P. Malcolm Rhodes and [2]Chris R. Balley, Departments of Microbiology and [1]Genetics, University of Leeds, Leeds, LS2.9JT, UK and [2]Celltech Ltd, 244-250 Bath Road, Slough, Berks, SL1.4DY, UK.

The usefulness of streptomycete plasmid cloning vectors, particularly in industrial applications, is dependant on their long term stability, preferably in the absence of antibiotic selection. The mycelial nature of streptomycetes makes it impossible to determine accurately the proportion of plasmid-containing vegetative cells by direct plating. Therefore, two alternative methods for measuring plasmid content were tested using *S.lividans* TK21 containing representatives of high (pIJ101 series) and low (SCP2* series) copy number plasmids. Plasmid segregation into single colony-forming units (cfus) as spores (produced without antibiotic selection) and protoplasts was measured and used to assess the plasmid content of the original cultures from which the single cfus were derived. Six replicate experiments showed that viability of the spores (given as a central value, calculated using two-way analysis of variance) ranged from 63% (pIJ303) to 17% (pIJ702). When the original culture was grown with antibiotic selection, plasmid segregation into viable spores approximated to 100% except for pIJ365 (82%). When the spores were derived from defined mixtures of plasmid-containing (pIJ702) and plasmid-free vegetative cells, the proportion containing plasmids accurately reflected the composition of the original mixture. Analysis of six replicate experiments using protoplasts revealed lower viabilities ($\leq$3.5%) than those obtained using spores; also, considerable plasmid loss occured during protoplast formation or regeneration. Plasmid segregation into viable protoplasts was 13-17% for the high copy number pIJ101 series plasmids and 59% for the low copy number plasmid pIJ922. Plasmid analysis of spores and copy number determinations, where appropriate, are currently being used to investigate plasmid instability in serial subcultures using solid media and in liquid batch and continuous culture.

CC 419 TN5096 AND TN5097: IS493 DERIVATIVES THAT FUNCTION AS TRANSPOSONS IN STREPTOMYCES, Patricia J. Solenberg, Molecular Genetics Research, Lilly Research Laboratories, Indianapolis, IN 46285

Tn5096 and Tn5097 are transposable elements constructed by inserting an apramycin resistance gene, aac(3)IV, into the StyI site of the S. lividans insertion element, IS493. Transposition of Tn5096 and Tn5097 was observed in S. griseofuscus. The elements transposed from both replicating and non-replicating plasmids and inserted into a variety of unique genomic locations.

CC 420 MOLECULAR ASPECTS OF whiB, A GENE REQUIRED FOR SPORULATION IN Streptomyces coelicolor. J. Soliveri, N.K. Davis and K.F. Chater, Department of Genetics, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

The whiB gene is needed at an intermediate stage during sporulation of aerial hyphae in Streptomyces coelicolor, such that they coil but do not form sporulation septa. We report the cloning of whiB in a low copy-number transmissible plasmid vector; the exploitation of vector transmissibility in complementation studies and mutagenesis with Tn4560; and the sequence and transcription mapping of whiB. The major transcript of whiB originates from a "consensus-like" promoter, and is absent from young cultures. Its deduced product is a 10 kDa protein with 39% charged residues and four cysteines. This protein is required specifically during sporulation; a deletion of the whole cloned region gives a typical whiB phenotype which is restored to normal morphology by the cloned whiB gene. A leu 74 --> pro change in a whiB point mutant gives a phenotype identical to that of the deletion mutant. The whiB gene of S. lividans differs by a single base from that of S. coelicolor, and specifies an identical protein.

CC 421 ISOLATION AND CHARACTERIZATION OF TWO AMYLASE GENES FROM STREPTOMYCES GRISEUS, Anna Soltyk, Phyllis Krygsman, Graham Henderson, Eva Walczyk, Loida Escote-Carlson, Ewa Plawinska, Gisela Soostmeyer and Lawrence Malek. Cangene Corporation, Mississauga, Ontario, Canada L4V 1T4.

Two amylase genes were isolated from an S. griseus (IMRU 3499) DNA library by hybridization to oligonucleotides derived from amylase consensus sequences. Each gene was capable of expressing extracellular amylolytic activity in Streptomyces lividans. The DNA sequence of one amylase gene contained an open reading frame of 577 amino acids which was homologous (76 to 78%) to the amylase precursors of Streptomyces venezuelae and Streptomyces limosus. The promoter regions of the amylase genes of S. griseus and S. limosus also contained blocks of extensive homology. Expression of amylase from its natural promoter was compared to expression from promoters of the S. limosus amylase or Streptomyces fradiae aminoglycoside phosphotransferase genes. Amino terminal sequencing of the secreted amylase indicated the presense of a 33-amino-acid signal peptide preceding the mature protein which could be replaced with the signal peptide of S. griseus protease B. The deletion of a portion of the amylase gene encoding the carboxy terminal-103 amino acids resulted in the secretion of a truncated 47 kDa form of the amylase that was completely active. Neither of the amylase genes characterized in this study were the same as the reported amylase gene from S. griseus (IMRU 3570).

CC 422 EXPRESSION AND SECRETION OF A HETEROLOGOUS PHOSPHOTRIESTERASE GENE WITH AND WITHOUT A SIGNAL SEQUENCE IN STREPTOMYCES LIVIDANS. M.K. Speedie, S. Rowland, H-L. Zhang, V. Thaker, and B.M. Pogell, Department of Biomedicinal Chemistry, University of Maryland, and the Maryland Biotechnology Institute, Baltimore, MD 21201

A gene (opd) for a Flavobacterium membrane-bound phosphotriesterase has been cloned into the BglII site on pIJ702. When the resulting plasmid was transformed into S. lividans 66, the recombinant organism yielded high level extracellular production of the enzyme. In order to study the mode of export of the protein, homogenous protein was sequenced from the N-terminal end. The results show that the N-terminus of the mature extracellular protein begins at amino acid 30 (serine) of the amino acid sequence predicted by the gene sequence, indicating the presence of a signal peptide. The same N-terminal serine was found in the pure Flavobacterium membrane enzyme by W. Mulbry and J. Karns (USDA). A construction was made in which the gene for a truncated opd gene, which lacked the signal sequence (from J. Karns), was cloned into the SphI site of pRYE6, a pIJ702 derivative which contains the opd promoter region but no structural gene. The cloning strategy involved the use of a synthetic EcoRI-SphI adapter to link the truncated gene to the promoter. The resulting gene sequence should produce a protein with four additional amino acids at the N-terminus. If the signal sequence was required for secretion in S. lividans, then the enzyme should accumulate intracellularly. Growth experiments showed low levels of both intracellular and extracellular enzyme activity, suggesting that the signal sequence, gene expression and protein export are linked in this organism.

CC 423 LYSOGENY IN STREPTOMYCES VENEZUELAE: PROPHAGE SV5 IS STABLY MAINTAINED WITHOUT GIVING SUPERINFECTION IMMUNITY, Colin Stuttard and Karin Hahn, Department of Microbiology, Dalhousie University, Halifax, NS, Canada, B3H 4H7.

Streptomyces venezuelae strain 10712 harbors prophage SV5 in a cryptic, but apparently non-defective form; intact SV5 phages were released only under special conditions including recovery from proplast regeneration, and infection by heterologous phage. However, this lysogenic host was simultaneously sensitive to lytic infection by free SV5. Infection with broad or narrow host-range phages (S27 and VWB, respectively), caused induction of prophage SV5 in strain 10712 and all of its derivatives except mutant VS258. Infection with the generalized transducing phage SV1 did not induce SV5, nor did UV irradiation. Molecular hybridization tests confirmed the presence of SV5 DNA (about 35.5 kb) within chromosomal DNA of all 10712 derivatives, including VS258, and showed SV5 DNA did not hybridize with the giant linear plasmid of S. venezuelae, nor with genomic DNA of related strains 13s and ETH14630, or S. phaeochromogenes. No sequence similarities were detected between the DNAs of S27 or VWB and SV5, although some hybridization was detected between SV5 and SV1 DNAs. SV5 and VWB had cohesive-ended DNAs while S27 apparently did not. A superficially similar "archived" state of prophage P22 exists in Salmonella; it differs from the SV5 state in several respects, particularly in its induction by adenine starvation. The possibility that DNA rearrangement may be involved in the regulation of SV5 lysogeny in S. venezuelae is being investigated.

CC 424 ON THE ORGANIZATION AND EXPRESSION OF *TUF*-GENES IN *STREPTOMYCES* SPECIES, **E. Vijgenboom, B. Woudt, M. Verdurmen, G. van Wezel, P. Heinstra and L. Bosch, Department of Biochemistry, Leiden University, Leiden, The Netherlands.** Many efforts have been put into studying the organization and expression of *tuf*-genes in the bacterium *E.coli* encoding elongation factors necessary in protein biosynthesis. These studies have revealed a complex organization of several *tuf*-genes in independently regulated operons. Recently, studies were initiated to probe the organization and expression of homologous *tuf*-genes in *Streptomyces* species. At the moment, several of these candidate genes have been cloned and in some cases heterologously expressed. Furthermore, we will show our recent advances in the isolation and characterization of *tuf*-gene products from *Streptomyces* species.

CC 425 SUICIDE VECTOR SYSTEMS FOR STREPTOMYCETES, Wolfgang Wohlleben, Günter Muth, Bernhard Nußbaumer, Doris Hillemann and Alfred Pühler, Faculty of Biology, University of Bielefeld, D-4800 Bielefeld 1, F.R.G.

Replication of the *Streptomyces ghanaensis* plasmid pSG5 was shown to be temperature-sensitive (ts). The pSG5 replicon is stably inherited at temperatures below 34°C, while it is lost at incubation temperatures above 34°C. A family of cloning vectors was constructed, using the pSG5 minimal replicon and different marker genes. By increasing incubation temperature, these vectors can be eliminated from their host cells very efficiently. pSG5 vectors with cloned chromosomal fragments integrate via homologous recombination into the genome. Fragment specific integration (and mutation) could simply be selected after increasing temperature. This has been exploited for both, random and targeted mutagenesis.

The transformation efficiency for most of the commercially important Streptomycetes strains is too low to apply *E. coli* plasmids as non-replicative suicide vectors. Transformation frequency could be increased 10^2fold using single-stranded (ss) DNA. Therefore vectors with Streptomycetes marker genes containing either an origin of replication of ss-DNA phages or transferable *E. coli* plasmids carrying the mob-region of RP4 were constructed. Following transformation or crosses between the mobilizing *E. coli* strain S17-1 and Streptomycetes, respectively, transferred DNA could easily be integrated into the Streptomycetes chromosome. Whereas ts-plasmids are advantageous for primary isolation of mutants and adjacent DNA-fragments, the non-replicative vectors are suitable for creating stable and marked mutants.