MOLECULAR GENETIC CONTROLS OF MICROBIAL DIFFERENTIATION

Organizers: William E. Timberlake and Richard Losick

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Molecular Genetic Controls of Microbial Differentiation

Polarity Determination, Subcellular Morphogenesis, and Colony Growth

J 001 THE ROLE OF FTSZ IN BACTERIAL CELL DIVISION, Joe Lutkenhaus, Amit Mukherjee, Kang Dai, Erfei Bi, Jian Hwang, and Xunde Wang, Department of Microbiology, Molecular Genetics and Immuology, University of Kansas Medical Center, Kansas City, KS 66103.

The essential cell division protein FtsZ forms a ring at the future division site, designated the FtsZ ring[1]. During cell division the FtsZ ring decreases in diameter at the leading edge of the septal invagination. Upon completion of division the FtsZ is dispersed into the cytoplasm and is not associated with the new cell pole. Expression of inhibitors of division, SulA or MinCD, prevent localization which would explain their ability to block cell division. Under conditions where the block to division by SulA is released cells are able to resume FtsZ localization and division, even in the absence of protein synthesis. This argues that the action of SulA is completely reversible and that FtsZ and the signal for localization are completely stable. The localization of FtsZ was also examined in various fts mutants. Mutations in ftsZ result in a smooth filamentation phenotype and prevent FtsZ localization. In contrast, filaments formed due to mutations in ftsA or ftsI have an idented filamentation phenotype. In these filaments some FtsZ rings are observed, however, there is not one for each division site. This result suggests that in these mutants the FtsZ ring forms and initiates division but it's progression is blocked by the lack of FtsA or FtsI which results in ring instability. Recently it has been determined that FtsZ has a unusual GTPase activity[2]. It will be of interest to determine what role this GTPase activity has in the dynamics of FtsZ localization during the cell cycle.

[1]Bi, E., and Lutkenhaus, J. (1991) Nature **354**, 161-164.
[2]Mukherjee, A., Dai, K., and Lutkenhaus, J. (1992) Proc. Natl. Acad. Sci. USA (in press)

Chemotaxis and Motility

J 002 THE GENETICS OF GLIDING MOTILITY IN *MYXOCOCCUS XANTHUS*, Patricia L. Hartzell, Spencer MacNeil and Frederico Calara, Department of Microbiology and Molecular Genetics, University of California, Los Angeles.

Gliding is the directed motion of cells across surfaces that occurs in the absence of external organelles such as flagella. A diverse group of environmentally important organisms, including the myxobacteria, *Beggiotoa*, the cytophaga, and cyanobacteria, move by gliding. The most extensive genetic analysis of gliding has come from studies of *Myxococcus xanthus*, the behaviorally complex Gram-negative bacterium. Most nonmotile *M. xanthus* mutants carry two mutations (A$^-$ and S$^-$). Single mutations at only one locus, *mglA*, abolish motility, and define the only gene required for both A- (adventurous) and S- (social) motility. The *mgl* operon contains two co-transcribed open reading frames. The products of these ORFs are predicted to be proteins 18kDa and 22kDa. The majority of *mgl* mutations affect the downstream ORF, *mglA*. The predicted sequence of the MglA protein includes a near consensus GTP-binding site and has 34% identity with *S. cerevisiae* SAR1 protein over a 120 amino acid stretch. We have isolated an allele-specific second-site revertant of *mglA8*, a nonmotile mutant that makes MglA protein. Our data suggest that this suppressor identifies a protein that interacts with MglA.

To determine if *mglA* affects the transcription of other motility genes, we have isolated Tn*5-lac* insertions in the genes that affect motility. These insertions represent the first comprehensive collection of reporter-gene insertions in motility genes and are being used to study regulation and to characterize motility genes. Comparisons of these insertions in wild-type and *mglA* genetic backgrounds confirm that *mglA* does not regulate other motility genes globally.

We have found an *mglA* homolog in a number of flagellated bacteria, including *Salmonella typhimurium*, *Serratia marcescens*, and *Proteus vulgaris*. Our discovery of *mglA* in *S. marcescens*, together with recent reports that *S. marcescens* can glide in the absence of flagella, suggests that *mglA* controls gliding in this organism. Rabbit anti-MglA antibody cross-reacts with a protein in extracts of *S. typhimurium*, *S. marcescens*, and *Proteus vulgaris* that is approximately the same size as the *M. xanthus* MglA protein. We have shown that oligonucleotides that specifically recognize *mglA* from *M. xanthus* amplify *mglA* homologs from each of these organisms after PCR. These results suggest that the MglA protein is highly conserved among diverse bacterial species. Gliding motility, like swimming motility, should enable bacterial pathogens to infect their hosts more successfully. MglA protein may play a central role in this type of motility in flagellated organisms.

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O'Rear, J., L. Alberti and R.M. Harshey. 1992. Swarming Motility in *Serratia marcescens* 274 Requires Some Translocation System in Addition to a Functional Chemotaxis System. J. Bacteriol. J. Bacteriol. **174**:6125-6137.

J 003 MOLECULAR GENETIC APPROACHES TO *DICTYOSTELIUM* MOTILITY, Thomas T. Egelhoff, Randall J. Lee, Bruce Patterson, Kathleen M. Ruppel, Taro Q.P. Uyeda, and James A. Spudich, Departments of Biochemistry and Developmental Biology, Stanford University School of Medicine Stanford, CA 94305.

Myosin is a ubiquitous eukaryotic molecular motor that moves actin filaments in an ATP-dependent manner. Multiple approaches have been used to implicate myosin in a variety of nonmuscle movements such as cytokinesis, cell migration, capping of cell surface receptors, intracellular vesicle movement, and developmentally-associated morphogenetic shape changes. We are using the simple eukaryote *Dictyostelium discoideum* to further explore the role that myosin plays in these motile behaviors. *Dictyostelium* cells lacking the endogenous myosin heavy chain gene (created by gene replacement) exhibit striking defects in several types of cellular motility. This mutant background is used as a recipient for altered myosin heavy chain genes, and the effects of each introduced change on *in vivo* and *in vitro* myosin function are assayed. Various mutagenesis techniques have been employed to alter either the head of myosin (Subfragment-1, or S-1),which is the motor unit of the molecule, or the alpha-helical coiled-coil tail, which governs assembly of myosin into thick filaments.

Analysis of the tail domain of myosin has included serial truncations to determine regions of the tail necessary for filament assembly, as well as site-directed mutagenesis of phosphorylatable residues that have been implicated in control of assembly. Truncation studies have mapped the region of the tail necessary to drive filament formation *in vitro* to within 33 amino acids. Myosins bearing tails which are truncated N-terminal to this region are not competent to assemble, and are not able to complement the myosin-specific defects of the null strain, whereas myosins with tails truncated C-terminal to this domain are able to form filaments *in vivo* and *in vitro*. Site-directed mutagenesis studies have explored the consequences of converting phosphorylatable threonine residues either to alanine residues, thereby eliminating phosphorylation at these positions, or to aspartate residues, which mimics the negative charge state of the phosphorylated molecule at these positions. Replacement of the phosphorylatable threonines with alanines does not effect the *in vitro* assembly of the myosin, but results in substantial overassembly of the myosin into the cytoskeleton *in vivo*. Cells are still able to use this myosin to drive capping of cell surface receptors, cytokinesis, and morphological changes during development. Replacement of the phosphorylatable threonines with aspartate eliminates filament assembly *in vitro* and renders the myosin unable to drive any tested contractile events *in vivo*. These results demonstrate that heavy chain phosphorylation plays a key modulatory role in controlling the recruitment and assembly of myosin within the cell.

Site-directed mutagenesis of the motor domain initially explored regions thought to be important for ATP and actin interactions. This approach yielded mutated myosins that are no longer able to bind nucleotide but which bind actin tightly (rigor binders), myosins that have a decresed affinity for nucleotide or decreased ATP turnover, and myosins with decreased stability *in vivo* and *in vitro*. *In vivo* effects of these various mutations ranged from slight temperature sensitivity to a fully null phenotype, depending upon the severity of the biochemical defect. Recent efforts have involved creating banks of random point mutations over short stretches of highly conserved myosin sequence throughout the myosin head. Three general phenotypic classes have emerged from this approach: wild-type like cells (normal cytokinesis and development *in vivo*) that express myosins with no or only minor biochemical defects; intermediate cells (defective or unreliable cytokinesis and development *in vivo*) that express myosins with a variety of biochemical defects, including lowered actin-activation of ATP turnover and uncoupling of ATP hydrolysis and motility *in vitro*; and null-like cells (no cytokinesis and early developmental arrest *in vivo*) that express myosins that are specifically unable to hydrolyze ATP. In collaboration with Dr. Ivan Rayment (U. Wisconsin, Madison), we are currently trying to place each of these changes on the high resolution three-dimensional structure of S1 (recently determined by Dr. Rayment and his colleagues). We are also collaborating with Dr. Ken Holmes' group (Max-Planck-Institute, Heidelberg) and Dr. Dietmar Manstein (NIMR, Mill Hill, London) to analyze the position of these mutations in relation to the docked structure of S1 bound to actin in the actin-S1 complex formed in the absence of ATP. These studies should provide a framework for understanding how myosin links ATP hydrolysis to movement and force production, and how this motor function leads to motile activities *in vivo*.

J 004 MULTIPLE G PROTEIN-LINKED cAMP RECEPTORS CONTROL DEVELOPMENT IN *DICTYOSTELIUM*, Lijun Wu, Mike Caterina, Mei-Yu Chen, Dale Hereld, Robert Insall, Ji-Yun Kim, Pam Lilly, Jacqueline Milne, Carole Parent, Geoffrey Pitt, and Peter N. Devreotes, Dept. of Biological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205.

G protein-linked signal transduction pathways, which are basically the same as those in mammalian cells, play essential roles during the starvation-induced development of *Dictyostelium discoideum*, a simple eucaryotic organism. We have identified a family of genes encoding four cAMP receptors (cARs) that have been implicated in multiple aspects of development. All of these receptors contain seven putative transmembrane domains, a characteristic of receptors that are linked to G proteins. *Dictyostelium* possesses at least eight G protein α-subunits, each of them is transiently expressed at a distinct stage of the developmental program. These eight G protein α-subunits are 35-50% identical to each other and to their mammalian counterparts but do not seem to fall into any obvious subtypes. In addition, a β-subunit, which is presumably involved in the formation of heterotrimers with each of the α-subunits, has been cloned. The β-subunit is constitutively expressed during growth and development and it is about 70% identical to those in higher eucaryotes. It has been shown that Gα2 is responsible for many receptor-mediated responses such as chemotaxis, activation of PLC, and gene expression. However, gα2$^-$ cells can still carry out cAMP-stimulated Ca^{++} influx and Gα2 phosphorylation, suggesting that other G proteins may be involved in these processes. gα7$^-$ and gα8$^-$ cells, generated by gene disruption, do not exhibit any obvious defects during growth and development, indicating that either some α-subunits are functionally redundant or the defects are too subtle to be detected under our standard laboratory conditions. Moreover, genes for two adenylyl cyclases have been identified in *Dictyostelium*. One of them, responsible for cell-cell signaling in aggregation, resembles its mammalian counterpart in topological structure.

J 005 THE "*FRIZZY*" GENES OF *MYXOCOCCUS XANTHUS* CONTROL DIRECTED MOTILITY, David R. Zusman, Gonzalo Acuna, Eldie Berger, Thilo Kohler, Mark McBride, Wenyuan Shi, Karen Smith, and Katherine Trudeau, Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley CA 94720.

Myxococcus xanthus, a Gram-negative, non-flagellated gliding bacterium, exhibits multicellular interactions during vegetative growth and fruiting body formation. A class of non-fruiting mutants was isolated that formed tangled, frizzy filaments under fruiting conditions. The frizzy (*frz*) genes were found to be required for controlling directed motility by causing cells to periodically reverse their direction of movement. We have cloned and sequenced the *frz* genes and found that they encode proteins that are homologous to all of the major enteric chemotaxis proteins, with the exception of CheZ. For example, FrzA is homologous to CheW, FrzE is homologous to both CheA and CheY, FrzF is homologous to CheR, FrzG is homologous to CheB, and FrzCD is homologous to the methyl accepting chemotaxis proteins (MCPs). Despite these similarities, fundamental differences exist between the myxobacteria and the enteric bacteria in terms of the ability of cells to recognize and respond to substances in their environment. Since chemical stimuli had not been characterized in *M. xanthus*, we used FrzCD and its ability to be methylated or demethylated to search for substances which were recognized by *M. xanthus*. Some of these stimuli were found to have profound effects on the behavior of cells. We have used these stimuli to establish the first clear-cut data demonstrating chemo-attraction to nutrients and chemo-repulsion to repellents in this organism. Chemotaxis did not occur in the *frz* mutants. We found that the pattern of gliding motility and chemotaxis of the cells was correlated to the methylation pattern of FrzCD. Since the methylation of FrzCD is most striking during developmental aggregation, we hope to use this assay to identify aggregation-related stimuli during development.

Sex Determination and Mating Factors

J 006 GENETIC DETERMINANTS OF FILAMENTOUS GROWTH AND TUMOR INDUCTION OF THE CORN SMUT FUNGUS *USTILAGO MAYDIS*. Flora Banuett and Ira Herskowitz. Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

The smut fungus *Ustilago maydis* infects corn and induces tumors. *U. maydis* is a dimorphic basidiomycetous fungus: the unicellular haploid form is yeast-like and divides by budding; it is non-pathogenic and saprophytic (grows on all kinds of substrates); the filamentous dikaryotic form is pathogenic and parasitic (needs the plant for its growth). The dimorphic transition that characterizes the life cycle of *U. maydis* is governed by two mating type loci, *a* and *b*. Fusion of two haploid cells carrying different *a* and *b* alleles results in formation of the pathogenic filamentous dikaryon. Genetic analysis (reviewed in Banuett, 1992) indicates that *a* and *b* control independent and overlapping steps in pathogenic development: different *a* alleles are necessary for establishment of the dikaryon; different *b* alleles are necessary for maintenance of the pathogenic state; different *a* and different *b* allales are necessary for maintenance of filamentous growth. The *a* locus has two naturally occurring alleles *a*1 and *a*2 (Rowell and DeVay, 1954). Cloning and sequencing of the *a* locus (reviewed in Banuett, 1992) indicates that *a* encodes components of a pheromone response pathway -- each allele contains a pheromone precursor gene and a receptor gene. Thus, the *a* locus may control cell fusion. The *b* locus is multiallelic -- there are estimated to be 25 naturally occurring alleles (Rowell and DeVay, 1954). Given that different *b* alleles are necessary for filamentous growth and tumor induction, how does the dikaryotic fungal cell monitor identical from non-identical *b* alleles? The *b* locus contains 2 inseparable genes, *bW* and *bE*, each of which appears to be multiallelic (reviewed in Banuett, 1992). Each gene encodes a polypeptide that contains a homeodomain. There is no similarity at the amino acid level between the bW and bE polypeptides. It is hypothesized that bW and bE monomers contributed by different *b* alleles interact to form a heromultimeric regulatory protein (for example, bW1-bE3 or bW4-bE7) that governs target genes for filamentous growth, tumor induction and saprophytic growth. Putative target genes for the *b* loucs have been identified. Molecular genetic analysis of some of these genes will be presented as well as identification of a gene downstream of the receptor gene.

Banuett, F. (1992) *Ustilago maydis*, the delghtful blight. Trends Genet. 8: 174-183.
Rowell, J.B. and DeVay, J.E. (1954) Genetics of *Ustilago zeae* in relationship to basic problems of its pathogenicity. Phytopathology 44, 356-362.

J 007 THE CONTROL OF MATING AND DEVELOPMENT IN USTILAGO MAYDIS. R.Schlesinger[1], J.Bergemann[1], F.Schauwecker[2], M.Bölker[2], M.Urban[2], B.Schroeer[1], and R.Kahmann[2], [1]Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, D-1000 Berlin 33 and [2]Institut für Genetik und Mikrobiologie, Maria-Ward-Str.1a, D-8000 München 19, Germany.

In the maize pathogen Ustilago maydis mating and sexual development are governed by two mating type loci termed a and b. The a locus controls the fusion of haploid cells and exists in two different alleles, a1 and a2. Both encode a precursor of a specific mating pheromone and a receptor that recognizes the pheromone of opposite mating type. The multiallelic b locus controls pathogenicity and the sexual development of the fungus. Each b allele codes for two regulatory proteins (bW and bE) which both contain a homeodomain and function in pairwise combination. It has been proposed that dimerization of one bW and one bE polypeptide from different allelic origin leads to formation of an active regulatory species, which turns on development. The dimer formed by bW and bE derived from the same allele, however, is proposed to be inactive.
The formation of the dikaryon is accompanied by a morphological change from yeast-like to filamentous growth. The maintenance of the filamentous form is controlled by both the a and the b locus. We demonstrate that the expression of the pheromone genes is environmentally regulated and can be induced by starvation or by specific media. Pheromone expression is observed also in the dikaryotic stage indicating that the a locus participates in the regulation of filamentous growth by autokrine response of the pheromone receptors.
The function of the homeodomains of the b polypeptides has been studied by site directed mutagenesis. We infer from the results that the homeodomains of both proteins are required for function. Biochemical analysis of the b proteins has been initiated by expressing epitope tagged variants in E.coli. By immunoprecipitation we could demonstrate that the bW and bE proteins can form heterodimers in solution. Allelic and non-allelic combinations result in heterodimers suggesting that dimerization occurs via the constant regions of the proteins. This is consistent with our model which predicts that the critical step of self/non-self recognition occurs at the level of DNA binding and/or activity of the dimer. We also present an initial analysis of genes that are differentially expressed in the filamentous stage. These genes are candidates for b regulated genes and are potential targets for the b polypeptides.

J 008 DIRECTIONALITY AND CELL FATE DETERMINATION CONTROLS IN FISSION YEAST MATING-TYPE DETERMINATION, Amar Klar, Genevieve Thon, Jagmohan Singh, and Michael Bonaduce, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Laboratory of Eukaryotic Gene Expression, P.O. Box B, Building 539, Frederick, MD 21702

Any model of cellular differentiation must explain how a cell can produce two daughters with different developmental fates. Within a pair of sister cells of the fission yeast, only one cell is switching-competent as it generates one switched and the other unswitched daughter in 72% to 94% of cell divisions. Our work has demonstrated that the devleopmental asymmetry between sister cells is a consequence of inheriting the complementary, but nonequivalent, parental DNA chains of the *mat1* locus and is not due to unequal distribution of other nuclear or cytoplasmic factors.

mat1 switching is initiated by a site-specific double-stranded break (DSB) at *mat1*. The break is repaired by a gene conversion event, whereby a copy of the donor locus (*mat2-P* or *mat3-M*) is transmitted to *mat1*, resulting in a switch. We have proposed that the DSB is made perhaps during replication such that one of the two sister chromatids is cleaved. We found that *swi7*, a gene required to generate the break, encodes the catalytic subunit of DNA polymerase α. This result suggests that generation of the DSB is coupled to DNA replication. We propose that the act of DNA replication itself advances the developmental program in this sytem. This principle may be of general significance as it is applicable to any differentiating system.

The cells with the DSB mostly switch to the opposite mating type, suggesting that donor loci are used nonrandomly. We have shown that cells choose the specific donor regardless of its genetic content. Thus, directionality is regulated by a mechanism that selects particular donor locus. Furthermore, the donor loci are unexpressed, despite the fact that they have the promoter sequences. In addition, the 15 kb region between *mat2* and *mat3* is prohibited from crossing-over. Our working model is that a specific chromatin organization in the mating-type region promotes specific donor choice and inhibition of recombination as well as silencing the donor loci. We have obtained mutations in other genes which alter these controls.

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J 009 MATING IN NEUROSPORA CRASSA AND ITS RELATIVES, Robert L. Metzenberg and Thomas A. Randall, Dept. of Biomolecular Chemistry, University of Wisconsin, Madison WI 53706.

N. crassa exists in two mating types, designated A and a, and the two must come together under suitable conditions for mating to occur. The genetic region controlling this difference was cloned by S. Vollmer and C. Yanofsky. Work by them and by L. Glass, C. Staben, J. Grotelueschen, and R. Metzenberg has shown that these are not alternate forms of basically similar genetic information, but are totally unrelated in sequence. For this reason, we have called them idiomorphs, not alleles. Early results indicated that immediately beyond the idiomorphs in each direction, their flanking sequences became identical, so that the transition between non-resemblance and identity was absolute. We now find that a few hundred basepairs centromere-proximal to the borders between the idiomorphs and their flanking sequences, the two begin to diverge again so that they are about 10-20% dissimilar for about an additional 1000 basepairs. Several transcripts originate in this region of moderate dissimilarity, and several lines of evidence suggest that they are concerned with mating. One of the transcripts that maps to the centromere-proximal flank of the A idiomorph has an open reading frame of 38 amino acids terminated at the carboxyl end with a -CAAX motif appropriate for farnesylation, and the inferred peptide shows homology to other known or inferred mating pheromones. No DNA homologous to this region can be seen in any of the extant homothallic species of Neurospora, nor in the A mating type of most of the pseudohomothallic strains.

Cell Cycle Controls in Cellular Differentiation

J 010 THE MOLECULAR GENETICS OF NUCLEAR MIGRATION IN *ASPERGILLUS NIDULANS*, N. Ronald Morris[1], Stephen A. Osmani[2], Xin Xiang[1] and Ya-hui Chui[1], [1]Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J.,[2]Geisinger Clinic, Danville, PA.

Nuclear migration plays a fundamental role in many developmental processes in both higher and lower eukaryotes. *Aspergillus nidulans* is a particularly suitable organism for the study of nuclear migration, because nuclei migrate actively in the fungal mycelium. Mycelial nuclear migration is known to require functional microtubules: benomyl, an antimicrotubule agent, and mutations in tubulin inhibit nuclear migration in *A. nidulans* [Oakley & Morris, Cell 19(1980)255; Oakley & Morris, J. Cell Biol. 96(1983)1155]. To identify other components of the machinery responsible for nuclear migration and the signalling system that specifies when nuclei are to move, we have undertaken a search for temperature sensitive (ts) mutants in which nuclear distribution along the mycelium is abnormal. Three ts, non tubulin genes have so far been identified, *nudA*, *nudC* and *nudF*. Strains carrying ts mutations in these genes conidiate poorly at permissive temperature. Conidia produced at permissive temperature can germinate at restrictive temperature, but only form short germlings and are unable to form colonies. DAPI staining of conidia germinated at restrictive temperature showed that the nuclei are able to divide but not to migrate. The *nudC* gene has been cloned and found to encode a 22kD protein whose sequence provides no clues to its function [Osmani et al. J.Cell Biol. 111(1990)543. We have undertaken a search for extragenic suppressors of *nudC* to identify interacting genes that may help us determine its function. The *nudF* cDNA was initially identified as an extragenic suppressor of *nudC*. One extra copy of *nudF*, when transformed into a *nudC* mutant, suppresses the *nudC* temperature sensitivity for growth. The derived amino acid sequence of *nudF* resembles a tripartite G-protein β subunit.

J 011 A SIGNAL TRANSDUCTION PATHWAY COUPLING CELL DIVISION AND POLAR MORPHOGENESIS IN *CAULOBACTER CRESCENTUS*, Austin Newton, Gregory Hecht, Todd Lane, Elizabeth Ninfa, Noriko Ohta, and Jurg Sommer, Princeton University, Princeton, NJ 08544.

We have proposed that the temporal and spatial cues required for the precisely-controlled sequence of developmental events leading to formation of the new swarmer cell in *C. crescentus* and its subsequent differentiation into a stalked cell are provided by steps in the underlying cell division cycle (1). Previous work had shown that ongoing chromosome replication is required for flagellum biosynthesis and completion of steps in the cell division pathway are required for gain of motility, stalk formation, and pili assembly. More recently, a pseudoreversion analysis of *pleC* mutants that are defective in assembly of active flagella and stalk formation has furnished direct genetic evidence that the developmental and cell division cycle pathways are interconnected (2). Outside suppressors of *pleC* that compensated for the motility defect also conferred a cold sensitive cell division defect and mapped to the three new cell division genes, *divJ*, *divK* and *divL*. Nucleotide sequence analysis now suggests that the products of these genes are members of a signal transduction pathway coupling polar morphogenesis to events in the cell cycle. The *divJ* gene encodes a predicted protein with an extensive hydrophobic N-terminal region and a C-terminal domain that contains the invariant residues belonging to a family of bacterial sensor proteins with histidine protein kinase activity (3). DNA fragments complementing the bypass suppressors in *divK* encode a 125 residue polypeptide containing the structural element conserved in the amino-terminal domains of response regulators like CheY. Recently, Ely and coworkers have shown the *pleC*, like *divJ*, encodes a protein with a C-terminal histidine protein kinase domain. We propose that DivJ and PleC are membrane-associated protein kinases, which along with response regulator DivK, and perhaps other uncharacterized gene products like DivL, are members of a complex signal transduction pathway regulating both the cell cycle and differentiation in *Caulobacter*. Thus, protein modification by phosphorylation may play a central role in coupling developmental events to progress through the cell division cycle.

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2. Sommer, J.M. & Newton, A. (1991) Genetics **129**, 623-630.
3. Ohta, *et al.*, (1992) Proc. Natl. Acad. Sci. **89**, 10297-10301.

Multicellular Development

J 012 CELL INTERACTIONS IN *MYXOCOCCUS XANTHUS* DEVELOPMENT, Dale Kaiser, Stanford University, Stanford, CA

Starvation leads to aggregation of about 100,000 cells in a fruiting body, which ultimately becomes filled with dormant myxospores. Random transcriptional fusions to lacZ provide markers of developmental gene expression: at least 30 genes increase their activity according to a regular time schedule. The *Myxococcus* program of fruiting body development combines differential gene expression with morphological change. Different cell to cell signals are implicated by four complementary classes of developmental mutants. Mutants of the *asg*, *bsg*, *csg*, and *dsg* classes cannot sporulate when they are alone, but their sporulation can be rescued by co-development with wild-type cells in cell mixtures (chimeras). Each class of nonautonomous mutants affects the developmental program in a different way. The chemical identification of the *asg* and *csg* signal molecules from wild-type cells shows that these two classes are deficient in production of different signals.

Development can be restored to *asg* mutants by the addition of conditioned medium in which wild-type cells had been developing, or of A-factor purified from conditioned medium. A-factor, which is found in conditioned medium in proportion to the cell density, is any one of a set of 6 amino acids or combination of the 6. As decreasing amounts of A-factor are added, the developmental response of *asg* mutant cells decreases. Wild-type cells fail to develop when their density is decreased below the point at which the level of A-factor is predicted to be limiting. The development of low-density *asg*$^+$ cells, like *asg* mutants, can be restored by addition of A-factor. Apparently, A-factor is used by *Myxococcus* to specify the minimum cell density required for the initiation of development.

Development can be restored to *csg* mutants by the addition of an extract of developing wild-type cells. C-factor has been purified from a sonic extract; it is a 17 kDa hydrophobic protein, the product of the *csg*A gene. C-factor requires proper cell alignment for signaling. Proper alignment is normally provided by the program of cell movement. C-factor signals continued aggregation and it initiates sporulation when cells have reached their proper arrangement in a nascent fruiting body. Myxobacteria appear to use both A and C signals to check sequentially the quality of cell-cell arrangements at different stages during fruiting body development.

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J 013 MULTICELLULAR FACTORS AFFECTING GENE EXPRESSION PATTERNS IN *E. COLI* COLONIES, James A. Shapiro, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637.

Gene expression patterns can be observed in colonies of bacteria containing stable *lacZ* fusion constructs on XGal indicator agar. Each fusion displays its own characteristic pattern. Even loci which do not show regulation in liquid culture experiments, such as *polA*, can be seen to be differentially expressed in colonies. The staining patterns result from differential LacZ expression organized into concentric rings and horizontal strata composed of cells that are not clonally related to each other. Such non-clonal organization of LacZ expression parallels the distribution of groups of morphologically distinguishable cell types within the colony. The existence of these coherent, non-clonal, phenotypically distinct zones within the colony indicates that the component cells are responding similarly to a set of external signals. Further evidence for long-range signalling can be obtained by following the responses of patterned LacZ expression to perturbations of normal colony development. Disturbance of colony expansion by glass fibers shows that coordinating signals extend a certain distance over the agar substrate before they attenuate. Interestingly, the response to these signals does not appear to be linear with distance from the colony perimeter. Encounters of colonies of different ages show that LacZ expression patterns can be synchronized, presumably by diffusible signals. Examination of XGal staining in sectors which arise on *lacZ* fusion colonies indicates that genetic changes do not always affect expression in each concentric ring in the same way. This observation suggests that there is multifactorial genetic control over LacZ expression from a particular fusion.

J 014 TIMING OF MYXOCOCCUS XANTHUS DEVELOPMENT, Lawrence J. Shimkets, Bheong-Uk Lee, and Keesoo Lee. Department of Microbiology, University of Georgia, Athens, GA 30602-2605.

The CsgA gene product, the C-signal, is a novel type of developmental timer whose extracellular concentration rises steadily during fruiting body formation of the myxobacterium *Myxococcus xanthus* and induces successive developmental stages at successively higher concentrations. The first objective of this work is to learn how *csgA* expression is regulated during development to achieve the observed gradual increase in extracellular CsgA. A variety of sensory information is processed in the *csgA* regulatory region including carbon, nitrogen, and phosphate availability, which inhibit expression, as well as peptidoglycan, motility, the B-signal, and the C-signal, which stimulate expression. To begin defining the DNA binding sites for the regulatory proteins in these signaling pathways nested deletions were constructed across the upstream regulatory region. Deletion of certain sequences over 400 bp upstream from the start site of transcription resulted in constitutive *csgA* expression suggesting the presence of a binding site for a negative regulator of *csgA* expression. Deletion of sequences 336 to 400 bp upstream eliminated expression suggesting that this fragment contains the binding site(s) for a positive regulator of *csgA* expression. Genetic and physiological factors regulating *csgA* expression were also studied by observing expression in different genetic backgrounds. Expression was eliminated in *csgA* and nonmotile strains suggesting that CsgA- and motility-sensing pathways activate gene expression in the -400 to -336 region (referred to as auto-activation). Auto-activation appears to be determined by the intracellular CsgA concentration rather than the extracellular concentration. These results suggest a model in which induction of *csgA* expression creates an autocatalytic loop resulting in increasing levels of CsgA and commitment to development. This loop may be broken by addition of high nutrient or inhibition of motility, both of which are known to result in premature cessation of development.

The second objective of this work is to learn how extracellular CsgA is perceived by cells and used to regulate developmental gene expression. CsgA has significant amino acid identity to a family of proteins which include some short chain alcohol dehydrogenases, although the functions of the majority of the members remain to be determined. One of the family members, the mouse Ap27 protein, is essential for induction of adipogenesis although the mechanism is unknown. An approach used to study the mechanism of CsgA perception involves the isolation of second site suppressor mutations which restore sporulation to *csgA* cells. One suppressor locus will be described which has been studied in considerable detail.

Spore Formation

J 015 GENE EXPRESSION AND ITS MORPHOGENETIC CONSEQUENCES DURING SPORULATION IN *STREPTOMYCES COELICOLOR*, Keith Chater, Paul Brian, Gary Brown, Celia Bruton, Kitty Plaskitt, Jamie Ryding, Juan Soliveri and Huarong Tan, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

Streptomyces coelicolor grows as a branching mycelium. Reproduction is by the formation of aerial hyphae, which become subdivided by regularly spaced, specialized septa and so develop into chains of spores. Four genes - *whiA*, *B*, *G* and *H* - have been identified that are needed both for sporulation septation and for the production of the grey pigment associated with normal spores. The *whiG* product resembles σ factors, particularly those that transcribe motility genes in other bacteria (σ^D of bacilli, σ^F of coliforms). Two *whiG*-dependent promoters have appropriate resemblances to regions conserved among promoters recognised by σ^D or σ^F. Increased levels of σ^{WhiG} cause sporulation in the substrate mycelium, suggesting that sporulation is not dependent on aerial mycelium formation in a very complex manner, and that transcription of many other sporulation genes may depend directly or indirectly on *whiG*. This has been investigated for the *whiB* promoter region and for promoters controlling the clustered biosynthetic genes for grey spore pigment. Surprisingly, none of these promoters appeared to be markedly reduced in activity in a *whiG* mutant; indeed, *whiB* transcription was not reduced in any developmental mutant tested.

The *whiB* gene product, a small protein with strongly acidic regions but a basic C-terminus, is thought to play a regulatory role, both because of its primary structure and because *whiB* mutants show severely reduced transcription not only of promoters for the spore pigment genes but also - unexpectedly - of the *whiG* promoter. Moreover, the same promoters also require the as yet uncharacterised *whiA* and *whiH* gene products for expression. In line with this common regulation, there are similar sequences in the various promoter regions.

This emerging understanding of the regulatory network for sporulation does not readily clarify the process of morphogenesis. In a first attempt to construct a testable hypothesis addressing this question, studies have begun on the metabolism of storage compounds, especially glycogen. Glycogen is deposited in two locations in colonies: in a broad zone at the junction of the substrate and aerial mycelium, and - transiently - in the sporulating parts of aerial hyphae. In the model, this not only provides localized depots for carbon skeletons, but also regulates the cytoplasmic osmotic pressure, influencing the movement of water from the base of aerial hyphae and perhaps also across the cell membrane. Decreased turgor pressure following condensation of glucose residues into glycogen would limit extension growth and facilitate septal ingrowth, whereas high turgor pressure generated following glycogen degradation would favour either extension growth in undifferentiated aerial hyphae or, in later stages, rounding up of cylindrical pre-spore compartments into spherical spores.

J 016 NOVEL DNA BINDING PROTEINS AND DNA STRUCTURE IN BACTERIAL SPORES, AND THEIR EFFECTS ON DNA PROPERTIES, Peter Setlow, Biochemistry Department, University of Connecticut, Farmington, CT 06030.

The DNA in dormant spores of Bacillus and Clostridium species is saturated with a novel group of DNA-binding proteins, termed α/β-type small, acid-soluble spore proteins (α/β-type SASP). These proteins are synthesized only in the developing spore beginning at the third hour of sporulation, and are degraded to amino acids in the first minutes of spore germination. There are multiple (up to seven) α/β-type SASP in any one species, with each protein coded for by a unique gene, all of which are scattered around the chromosome. The primary sequences of these proteins have been extremely highly conserved, both within and across species. However, their sequences show no obvious homology with any other class of protein or protein sequence motif. Studies in vivo have shown that there is sufficient α/β-type SASP in dormant spores to saturate the DNA, and that these proteins have marked effects on the apparent supercoiling, the UV photochemistry, and the stability of the DNA within the spore. Studies in vitro have shown that α/β-type SASP are relatively non-specific, double stranded DNA binding proteins, with a preference for DNAs (i.e. - polydG·polydC) which can most readily adopt an A-like conformation. In vitro studies using complexes of various DNAs with any of a number of α/β-type SASP have also duplicated the effects of these proteins on DNA supercoiling, UV photochemistry and stability which are seen in vivo. This in vitro work (in collaboration with Scott Mohr, Boston University) has further shown that α/β-type SASP binding causes DNA to change from a B-like to an A-like conformation, which presumably results in most of the changes in DNA properties noted above. The effect of the binding of this novel group of DNA-binding protein to spore DNA in vivo, is to render the spore's DNA much more resistant to UV irradiation, and more able to stably survive long periods of dormancy.

J 017 SHEDDING LIGHT ON CONIDIATION IN NEUROSPORA. Charles Yanofsky[1], Frank Lauter[1] Carl Yamashiro[1] and Daniel Ebbole[2]. [1]Stanford University, Stanford, CA. [2]Texas A and M, College Station, TX

The filamentous fungus Neurospora crassa undergoes an ordered developmental process leading to the production of multinucleate asexual spores, conidia. Desiccation, exposure to light, and other environmental cues, induce and/or stimulate a sequence of morphological events culminating in conidia formation. Mutants are known that are blocked at different steps in this developmental process. We are interested in determining the sequence of regulatory events that are responsible for conidiation. A set of so-called con genes were isolated to be used as regulatory targets; these genes are preferentially expressed at different stages of conidiation. V. Russo's group has isolated a set of Neurospora genes on the basis of their light responsiveness. Several of these blue light-inducible genes are also activated developmentally during conidiation, including eas, the structural gene for the major rodlet protein that coats the outer surface of conidia. Some of the con genes also are expressed along pathways leading to the formation of the other two types of spores, microconidia and ascospores. Functional dissection of the genetic segments of a clone containing one of the con genes, con-10, identified a repeat enhancer sequence, either copy of which promotes transcription during conidiation. Sites distinct from the enhancer also contribute to transcriptional activation. Transcription of con-10 in mycelium is markedly stimulated by light. This light response was found to be subject to day-night control, i.e., light activation was dependent upon prior growth in the dark. There is little or no expression in mycelium during continuous growth in the light or dark. Incubation in darkness following a light pulse results in circadian expression of the gene. Consistent with this observation, day-night control is altered in the circadian rhythm mutant, frq-9. Conidiation mutants that block developmental expression of con-10 do not affect its response to day-night control. Successive deletion of the con-10 upstream region increases con-10 expression in the light, suggesting that light may normally repress con-10 expression at one or more upstream sites. Using an appropriate selection, trans-acting mutants have been isolated that express con-10 abnormally, during mycelial growth. In some of these mutants other con genes also are transcriptionally active. One of these mutants was examined for day-night control of con-10 expression and it was observed that light did not repress normally. Together these findings indicate that at least some of the con genes are multi-responsive; they are turned on at different stages of conidiation, they are activated along three spore developmental pathways, and they respond to signals generated by development, light, and circadian rhythm.

Pathogenic and Symbiotic Mechanisms

J 018 ANALYSIS OF THE *ARABIDOPSIS* DEFENSE RESPONSE TO *PSEUDOMONAS* PATHOGENS, Frederick M. Ausubel, Jane Glazebrook, Jean Greenberg, Fumiaki Katagiri, Michael Mindrinos, and Guo-Liang Yu, Department of Genetics, Harvard Medical School, and, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

We have studied the response of *Arabidopsis thaliana* to the bacterial pathogen *Pseudomonas syringae* pv. *maculicola (Psm)* strain ES4326. Several previously unknown *Arabidopsis* defense-related genes were identified including ones encoding a glutathione-S-transferase, a superoxide dismutase, a lipoxygenase, and two calmodulin-like proteins. Interestingly, mRNA corresponding to each of these genes displayed markedly different patterns of accumulation during the defense response to *Psm* ES4326. We have isolated three categories of *Arabidopsis* mutants that show an aberrant defense response to *Psm* ES4326. Three mutants were isolated that do not mount a hypersensitive defense response (HR) when infiltrated with *Psm* ES4326 carrying the cloned avirulence gene *avrRpt2* but are still able to display an HR in response to other *avr* genes. At least two of these mutants are allelic and map to chromosome IV. Chromosome walking is underway to clone the gene that encodes the putative resistance gene corresponding to *avrRpt2*. To facilitate the identification of additional *Arabidopsis* mutants that do not mount an HR in response to an *avr* gene, we developed a novel method that involves vacuum infiltration of seedlings growing in petri plates. In this method, seedlings that respond with an HR to the *avr* gene are killed whereas mutants that cannot mount an HR survive. We also isolated three mutants that synthesize decreased levels of camalexin, an indole-based *Arabidopsis* phytoalexin. Two of the three camalexin mutants are significantly more permissive for the growth of *Psm* ES4326 than wild-type plants. Finally, five *Arabidopsis* mutants were isolated that display accelerated disease symptoms in response to *Psm* ES4326. These latter mutants, which were given the name *acd* for accelerated cell death, were assigned to two complementation groups.

Secondary Metabolism and Antibiotic Production

J 019 FUNCTIONAL AND EVOLUTIONARY RELATIONSHIPS OF GENES FOR ANTIBIOTIC BIOSYNTHESIS AND PRIMARY METABOLISM.
David A. Hopwood, John Innes Institute, Norwich, U.K.

Fatty acid synthases (FASs) and polyketide synthases (PKSs) of Type I are multifunctional proteins carrying a series of active sites for each of the sub-functions of carbon chain assembly and modification (acyl transfer, acyl carrier function, condensation, keto-reduction, dehydration and enoyl reduction); in contrast, Type II synthases are multi-enzyme complexes with separate polypeptides for each sub-function. Recently, the sequences of several genes or gene sets for actinomycete and fungal synthases have been determined (reviewed in Refs. 1 and 2). They include genes for fatty acid biosynthesis, a fundamental aspect of primary metabolism, and for the synthesis of the polyketide precursors of both secondary metabolites (antibiotics) and spore pigments, the latter being components of differentiated cells. These sequences have shown that earlier indications of common chemical mechanisms uniting fatty acid and polyketide synthesis reflect clear evolutionary relationships between the genes for the two classes of enzymes. Several other interesting points have emerged. (1) Among actinomycetes, there are examples of Type I and Type II organization for both FASs and PKSs. (2) In one and the same actinomycete, *Streptomyces coelicolor*, clusters of gene products for an antibiotic (actinorhodin) and for a spore pigment are not normally involved in cross-talk (mutations in each cluster uniquely affect either actinorhodin or spore pigment production), yet at least some of the gene products can functionally replace each other when their expression is artificially manipulated[3]. (3) The general organization of the *Penicillium patulum* PKS for 6-methylsalicylic acid resembles that of a vertebrate FAS, rather than that of the FAS of the fungus itself; in contrast, a *Brevibacterium ammoniagenes* FAS appears to be more closely related to the fungal FAS[4]. The significance of these and other phylogenetic and ontogenetic inter-relationships between primary and secondary metabolic genes is now being analysed by a combination of chemical and genetical approaches. One aim is to determine the basis for the "programming" of FAS and PKS enzymes that determines the chain length and the degree of reduction and branching of their products, and therefore their potential roles in primary or secondary metabolism and development. Another is to elucidate the mechanisms that lead to appropriate temporal and spatial expression of the genes for the various synthases, as primary metabolism gives way to secondary metabolism and eventually to differentiation into spores during the life cycle or the actinomycete or fungus.

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J 020 LIFE AND DEATH IN STATIONARY PHASE, Roberto Kolter, Marta Almirón, Gjalt Huisman, Sara Lazar, Peter Yorgey, and María Mercedes Zambrano, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

How does an *E. coli* cell remain viable when starved? What causes the death of a starved cell? During starvation *E. coli* cells enter a metabolically less active state, often produce extracellular peptide antibiotics, and become more resistant to a variety of environmental assaults. The physiology of the starved *E. coli* cell can be divided into three major stages: entry into stationary phase, the maintenance of cell viability during starvation, and exit from stationary phase. Different aspects of these stages are being addressed by research in our laboratory.

In response to the depletion of nutrients, *E. coli* cells alter the global pattern of gene expression and synthesize proteins needed for survival as well as peptide antibiotics that provide a competitive advantage under conditions of nutrient limitation. To understand the transcriptional regulatory mechanisms involved in the induction of these genes, we have analyzed several promoters induced by the cessation of growth. In vivo, many of these promoters show a strong dependence on the alternative σ factor, σ^S, the product of the *rpoS* gene. Even though σ^S is present in exponentially growing cells its activity increases during starvation. We have identified and are currently characterizing several genes that affect the activation of σ^S during starvation. The analysis of the synthesis of the peptide antibiotic Microcin B17 has provided us with some insights into the post-transcriptional and post-translational regulatory mechanisms at work in stationary phase cells.

While many cells in a culture die during starvation, some cells in the population can remain viable for months and years. We have isolated mutants that are unable to survive prolonged starvation. One such mutant, *E. coli surA*, has a defective cell wall that renders the cells more sensitve to some antibiotics and extremes in pH. We have also discovered a DNA binding protein, named Dps, which is synthesized during starvation and which appears to play both protective and regulatory roles in starved cells.

To study the properties of cells surviving prolonged incubation in stationary phase, we conducted experiments in which ten day-old (aged) cultures were mixed with one day-old (young) cultures of the same strain of *E. coli*. The results obtained indicate that stationary phase cultures of *E. coli* can be dynamic. Cultures undergo population shifts as mutants that are able to grow, take over the population. When aged and young stationary phase cultures were mixed, the cells from the aged culture grew, resulting in the death of the cells from the young culture. This capacity to take over a culture can result from mutations in the *rpoS* gene.

J 021 MULTIDOMAIN ENZYMES INVOLVED IN NON-RIBOSOMAL PEPTIDE SYNTHESIS, Mohamed A. Marahiel, Biochemie, FB Chemie, Philipps Universität Marburg, W-3550 Marburg, Germany

Biologically active peptides of linear and cyclic structures are synthesized non-ribosomally by several bacterial and fungal species by the aid of multifunctional enzymes that employ the thiotemplate mechanism. The constituents of the peptides, amino- and hydroxy acids, are activated as acyl adenylate before they are linked covalently as a carboxy thioester to the corresponding synthetase. The elongation of the peptide bound is catalyzed by the cofactor 4'-phosphopantetheine, which acts as an internal transport system. The completed peptide chain is then released from the multienzyme either by cyclization or maybe by the action of a specific thioesterase. Genes encoding multifunctional peptide synthetases have been isolated and were shown to be organized in large operons. The primary structures of the *tyc*-, *grs*- and *srf*- operons, which encode multienzymes for the synthesis of the cyclic peptide antibiotics tyrocidine, gramicidin S and surfactin, and that of the ACV-synthetase (a multienzyme that activates the condensation of the tripeptide precursor of penicillin G) have been determined. The analysis of these sequences revealed the organization of peptide synthetases in highly conserved domains. The aligned domains within the synthetases represent the functional units, which provide the template for peptide synthesis and their order determines the sequence of the peptide product. In *Bacillus* the genes encoding peptide synthetases (*tyc*, *grs*, *srf*) are induced at the level of transcription initiation during the transition from vegetative to stationary phase of growth. They are modulated by factors as diverse as the early sporulation gene product Spo0A, the transition state activator AbrB, a minor sigma factor and other gene products (ComA, ComP) required for the initiation of the competence pathway.

Secondary Metabolism and Antibiotic Production

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Environmental Sensing and Signal Transduction

J 022 TRANSCRIPTION FACTORS MEDIATING NITROGEN METABOLITE REPRESSION AND pH REGULATION IN ASPERGILLUS NIDULANS. Herbert N. Arst, Jr., Royal Postgraduate Medical School, London W12 ONN, UK

Nitrogen metabolite repression of the syntheses of enzymes and permeases involved in nitrogen nutrition, ensuring preferential utilisation of favoured nitrogen sources ammonium and glutamine, is mediated by the positive-acting product of the *are*A gene in *Aspergillus nidulans*[1,2]. *are*A controls expression of a large number (>100) of structural genes and the effects of *are*A mutations on expression of many of these are easily monitored by plate tests. Both the amino acid sequence of the 'zinc finger'- containing DNA-binding region and the nucleotide sequence to which the *are*A product binds place it clearly in the same class as the vertebrate GATA transcription factors. Using a combination of clones constructed *in vitro* and mutations obtained using classical genetics, it has been shown that large segments of the *are*A product are inessential for expression of most structural genes. Nevertheless segments inessential for expression of one or even a large majority of structural genes can be essential for expression of others[3]. Missense mutations in the DNA-binding region can have disparate effects on expression of different structural genes, including an elevation of expression of some concomitant with a diminution of expression of others[2,4]. Reversion of loss-of-function missense mutations in the DNA-binding region has produced an astonishing variety of phenotypes associated with mutational changes in the same or nearby codons. Unexpected sequence changes show, for example, that alterations in a 'zinc finger' loop residue can suppress a knuckle mutation, mutations in the conserved (amongst GATA factors) region C-terminal to the 'zinc finger' can suppress a loop mutation or a knuckle mutation and mutations in codons near to but outside the conserved DNA-binding region can suppress mutations within it.

A nidulans, a physiologically versatile organism, grows over a very wide pH range. Intracellular activities are protected from extremes of pH by the organism's pH homeostatic system. However, permeases, extracellular enzymes and secreted metabolites must function at ambient pH. The pH regulatory system of *A. nidulans*[5,6] ensures that such entities are synthesised only at pH values where they can function effectively (thus resulting, for example, in secretion of acid phosphatase in acidic media and alkaline phosphatase in alkaline media). A model consistent with all available data proposes that the *pac*C gene encodes a transcription factor which activates transcription of some structural genes whilst repressing transcription of others and that the *pal*A,B,C,E and F genes encode enzymes participating in synthesis of an effector molecule able to interact with and prevent both the positive and negative actions of the *pac*C product. The effector must be both present and in an active form at alkaline growth pH. The *pac*C gene has been cloned and sequenced. The derived amino acid sequence contains several 'zinc fingers' and a high frequency of the S/TPXX motif, found frequently in DNA-binding proteins.

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J 023 SIGNAL TRANSDUCTION AND DEVELOPMENT IN *BACILLUS SUBTILIS*. ALAN D. GROSSMAN*, KEITH IRETON, JOHN LEDEAUX, ROY MAGNUSON, DAVID RUDNER, KATHRYN JAACKS SIRANOSIAN, and JONATHAN SOLOMON. DEPARTMENT OF BIOLOGY, 56-510, MIT, CAMBRIDGE, MA 02139 PHONE: (617) 253-1515 FAX: (617) 253-8699

Mechanisms by which cells begin to differentiate into specialized cell types is intimately related to the detection and transduction of both internal and external signals and the alteration of gene expression in response to those stimuli. Our work focuses on the control of differentiation, gene expression, and signal transduction in *Bacillus subtilis*. Under appropriate conditions, cells of *B. subtilis* enter a developmental pathway that leads to the formation of dormant heat resistant endospores. Signals required for initiation of the sporulation pathway include nutrient deprivation, high cell density, and continued DNA synthesis. Spore formation is not the only developmental pathway available to *B. subtilis*. Under other conditions, a small fraction of cells in a culture develop the ability to be transformed with exogenous DNA. This state of genetic competence can occur when cultures reach high cell density. Several of the regulatory genes that control the initiation of sporulation also control the development of competence.

Our recent work has focused on several aspects of signal transduction and development. 1) Developmental gene expression and DNA synthesis are coupled during the initiation of sporulation. Treatments that disrupt DNA synthesis, either elongation or initiation, inhibit expression of genes that are activated by the *spo0A* gene product. The activity of the Spo0A transcription factor is regulated by phosphorylation and Spo0A and components of the phosphorylation pathway are required for early developmental gene expression. Mutations in *spo0A* which bypass the normal phosphorylation pathway also bypass the requirement for DNA synthesis, indicating that the DNA synthesis signals affect the phosphorylation pathway.

2) We have identified mutations in the Spo0A transcription factor that are active without phosphorylation (constitutively active). When expressed, these mutant proteins allow expression of developmental genes in the absence of the normal physiological signals (nutrient deprivation, cell density, DNA synthesis). The mutations encode in-frame deletions in the N-terminus of the protein, indicating that the normal function of the N-terminus is to inhibit the activity of the C-terminus.

3) The initiation of sporulation and development of genetic competence are regulated, at least in part, by extracellular signaling molecules that accumulate as cells reach high density. The *srfA* operon is required for genetic competence and is regulated by cell density. Response of *srfA* to cell density signals requires the products of the *spo0K* operon, an ATP-dependent transporter of oligopeptides that belongs to a family of conserved transport systems found in many organisms (the ABC transporters). The role of the *spo0K* system seems to be to sense extracellular signals and to activate pathways that lead to the phosphorylation (activation) of transcription factors necessary for competence and sporulation.

J 024 RECEPTOR/TRANSDUCER INTERACTION IN A PHOTOSENSORY SYSTEM, John L. Spudich and Elena N. Spudich, Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, TX 77030.

Sensory rhodopsin I (SR-I) is a 7-transmembrane helix receptor which mediates phototaxis in the archaebacterium *Halobacterium halobium*.[1] Biochemical studies of mutants defective in signaling have demonstrated a second intrinsic membrane protein transduces SR-I signals to a cytoplasmic signaling pathway.[2] This transducer, designated HtrI, consists of two transmembrane helices near its N-terminal followed by an extensive hydrophilic cytoplasmic domain, which contains a region homologous to the signaling and methylation domains of eubacterial chemotaxis transducers.[3] Physical proximity of HtrI to the chromophore (retinal) binding site of SR-I has been suggested by chromophore chemical linkage measurements. Here we report results from expression of the SR-I gene (*sopI*) in *H. halobium* in the presence and absence of HtrI, which indicate HtrI modulates photochemical reactions of SR-I. Expression of a synthetic *sopI* gene in an *htrI* $^{+}$*sopI* $^{-}$strain of *H. halobium* (obtained by targeted deletion of *sopI*) results in complete restoration of phototaxis responses (M.P. Krebs, E.N. Spudich, H.G. Khorana, and J.L. Spudich, in preparation). In membranes isolated from these transformants, SR-I photochemistry is normal; i.e., photoexcited SR-I produces in <1 msec a blue-shifted species (S_{373}) which decays thermally to the pre-stimulus state with a $t_{1/2}$ = 800 msec. S_{373} is the active signaling conformation of SR-I and its formation and decay require deprotonation and reprotonation, respectively, of the retinal attachment site in the photoactive center of the protein. The rates of these reactions are insensitive to the extra-membranous pH between pH 5 and 9. We have used the same expression plasmid to produce SR-I in an *htrI* $^{-}$*sopI* $^{-}$ deletion strain, Pho81. The absorption spectrum and flash-induced absorption difference spectrum of SR-I in membranes from transformed Pho81 are characteristic of those of SR-I in the presence of HtrI, indicating retinal binding and photochemical formation of S_{373}. However, the flash yield of S_{373} is 10% of that observed in the presence of HtrI. Further, the half-time of thermal decay of the S_{373} intermediate in the absence of HtrI is sensitive to external pH, increasing from ~100 msec at pH 5 to ~10 sec at pH 7. There is a nearly linear dependence of the first order $t_{1/2}$ for S_{373} decay on external proton concentration. In contrast, in the presence of HtrI coexpressed with SR-I in Pho81, the S_{373} decay halftime remains at ~800 msec throughout this pH range. These results can be understood in terms of a tight coupling between reactions occurring at the SR-I/HtrI interaction site and the proton transfer reactions occurring in the SR-I photoactive site.

[1]Spudich, J.L. and Bogomolni, R.A. (1984) Nature 312:509-513.
[2]Spudich, E.N., Takahashi, T., and Spudich, J.L. (1989) Proc. Natl. Acad. Sci. USA 86:7746-7750.
[3]Yao, V.J. and Spudich, J.L. (1992) Proc. Natl. Acad. Sci. USA *in press.*

Spatial Controls of Gene Expression

J 025 **GENETIC CONTROL OF *ASPERGILLUS NIDULANS* GROWTH AND DEVELOPMENT.** **Thomas H. Adams, Sang Tae Han, Jose Navarro, Bee Na Lee, and Jenny Weiser. Department of Biology, Texas A&M University, College Station, TX 77843.**

In contrast to many other cases of microbial development, *Aspergillus nidulans* conidiphore production initiates primarily as a programmed part of the lifecycle rather than as a response to nutrient deprivation. The molecular genetic mechanisms controlling activation of the conidiation pathway are largely unknown but lead ultimately to the expression of the *brlA* developmental regulatory locus which in turn results in activation of other genes required for conidiophore formation. We are addressing the question of what is responsible for regulated expression of *brlA* both through examining the *brlA* control region and through mutational analyses. The *brlA* locus consists of two overlapping transcription units, *brlA*α and *brlA*β, that encode functionally related polypeptides. *brlA*α is transcriptionally activated during development and *brlA* activity is required for full induction. In contrast, *brlA*β transcription is *brlA*-independent. *brlA*β mRNA is constitutively transcribed but translation of the *brlA* polypeptide is prevented by the presence of a short open reading frame (μORF) present in the 5' end of the *brlA*β mRNA. Removing the μORF inititiation codon leads to deregulated *brlA* expression and inappropriate activation of development. We have identified a large number of mutations that result in blocked or altered developmental activation of *brlA* expression. These mutations cause morphological abnormalities described as "fluffy" and are characterized by an unrestricted proliferation of aerial hyphae giving rise to large cotton-like colonies that, unlike wild type, are able to grow through other colonies. Fluffy mutants frequently produce relatively normal conidiophores from this hyphal mass but with a several day delay as compared to wild type. These mutants must therefore be capable of expressing the genetic machinery necessary for conidiophore development but are apparently altered in the ability to respond to the sig. .s that control normal developmental timing. One typical fluffy mutant results from mutation of the *acoD* gene. Strains containing a deletion of the *acoD* gene are completely aconidial and no *brlA* expression is detectable during growth on rich medium. However, this mutant phenotype can be partially remediated by limiting growth suggesting that unlike wild type strains, *acoD* null mutants develop in response to nutrient limitation. In addition, *acoD* mutant strains conidiate when grown in contact with either wild type colonies or with strains carrying different developmental mutations (e.g. *brlA*). A second fluffy mutant phenotype results from loss of function of the *flbA* gene. *flbA* mutants are not only fluffy but also have altered branching morphology and never conidiate under any circumstances. Sequence analysis of the *flbA* coding region indicates that the gene is predicted to encode a protein that shares significant similarity with the *Saccharomyces cerevisiae* SST2 product which is required for adaptation to mating pheromones. We propose that *acoD* is responsible for production of an extracellular factor that functions by directing growing cells to undergo development and that this response is mediated through a pathway involving both *flbA* and the products of other genes.

J 026 HETEROCYST DIFFERENTIATION IN CYANOBACTERIA, Robert Haselkorn, Jihong Liang and William J. Buikema, Dept of Molecular Genetics & Cell Biology, Univ of Chicago, Chicago Il 60637.

Heterocysts are cells specialized for nitrogen fixation that differentiate at regular intervals along the filaments of cyanobacteria. In *Anabaena* 7120, the interval of photosynthetic vegetative cells between pairs of heterocysts is approximately ten cells. The heterocyst is surrounded by a double-layered envelope outside its cell wall. This envelope limits the diffusion of gases, including oxygen, into the heterocyst. Together with changes in the photosynthetic apparatus that inactivate photosystem II, and the induction of respiratory systems that consume oxygen, the internal milieu of the heterocyst is made anaerobic. The heterocyst reduces nitrogen gas to ammonia, adds the ammonia to glutamate to make glutamine, and exports glutamine to neighboring vegetative cells. The latter continue to carry out photosynthesis and provide reduced carbon compounds to the heterocyst.

During growth on molecular nitrogen, differentiation of a vegetative cell into a mature heterocyst requires approximately the same time as the growth and division of a vegetative cell. The vegetative cell chosen for differentiation is the one located mid-way between two heterocysts. Thus the spacing pattern described above is maintained. The rules governing the selection of cells for differentiation, the nature of the signal molecules, and the identity of the proteins that respond to environmental cues in order to initiate development remain unknown. Since aerobic nitrogen fixation requires heterocyst differentiation, the selection of Nif mutants was expected to provide some that are defective in development. This expectation has been realized. We described previously a mutagenesis and selection protocol that led to the isolation of a mutation in the *hetR* gene. This mutation prevents initiation of heterocyst development and any expression of the *nif* genes. We describe here two other mutations that affect the pattern of heterocyst development in *Anabaena* 7120.

Mutant strain PAT-1 grows poorly, but does not die, under nitrogen-fixing conditions. This strain develops heterocysts almost exclusively at the ends of filaments. The heterocyst frequency is thus less than 10% that of wildtype *Anabaena*. However, mild sonication, which increases the heterocyst frequency by providing shorter filaments and therefore more ends, nevertheless fails to increase the rate of growth on molecular nitrogen. The filaments reduce acetylene as effectively as wildtype on a per heterocyst basis. The wildtype gene that complements the mutation in PAT-1, called *patA*, has been cloned and sequenced. The PatA protein, predicted from the gene sequence, contains 379 amino acids distributed among three domains based on predictions of hydropathy and flexibility. The carboxy-terminal domain is very similar to that of CheY and other response regulators in bacterial two-component environment-sensing systems. The PatA protein contains the highly conserved Asp residues that are phosphorylated by a histidine kinase in response to environmental cues in many other systems. Overexpression of the wildtype *hetR* gene, mentioned above, results in the production of strings of heterocysts, which is deleterious to growth. Mutation of the *patA* gene suppresses this phenotype, indicating that the PatA protein and the HetR protein are in the same regulatory circuit.

Mutant strain PAT-2 also grows poorly under nitrogen-fixing conditions. Heterocyst development is delayed by a day and eventually the filaments contain multiple heterocysts. The wildtype gene, *patB*, that complements the mutation in the PAT-2 strain, has been cloned and sequenced. The predicted protein is 60 kD and has two interesting domains. Near the amino terminus is a repeating pattern of Cys residues characteristic of ferredoxins with 4Fe-4S centers. Near the carboxy terminus there is a helix-turn-helix motif indicative of a DNA-binding domain. The mutation in the PAT-1 strain is a frame-shift that results in removal of the helix-turn-helix motif. It seems possible that PatB is a redox protein that functions as a repressor of differentiation at the level of gene transcription. The *patB* mutation does not suppress the multi-heterocyst phenotype due to overexpression of HetR, as might be expected. The *patA-patB* double mutant is a monster: under nitrogen-fixing conditions, the filaments have heterocysts only at their ends, but the vegetative cells increase in size as their distance from the heterocyst increases. The double mutant dies under these conditions.

J 027 CELL PATTERN FORMATION IN *ASPERGILLUS*, Bruce L. Miller, Karen Y. Miller and Jianguo Wu., Department of Biochemistry, University of Idaho, Moscow, ID 83843.

The formation of multi-cellular asexual reproductive structures requires the functional interaction of two groups of regulatory genes. One group (*brlA*, *abaA*, *wetA*) define a linearly regulated pathway required for formation of conidia while the second group (*stuA*, *medA*) are required for a restricted series of cell divisions that establishe the spatial organization of the conidiophore.[1,2] *stuA*$^-$ (stunted) mutants fail to undergo these division while *medA*$^-$ (medusa) mutants proliferate long branched chains of uninucleate cells and secondary conidiophore structures. During conidiation, StuA is required for the correct spatial expression, and MedA for the correct temporal expression, of *brlA* and *abaA*.[3] MedA and StuA are also required for development of the cleistothecium and associated nurse (Hulle) cells and for ascosporogenesis during sexual reproduction.

Developmental expression of StuA is regulated at both the transcriptional and translational level. Deletion analysis of upstream *cis*-acting DNA sequences revealed multiple elements required for normal expression of two alternatively processed *stuA* mRNAs in competent, undifferentiated hyphae and in developmental cultures. In addition, the peptide product of a mORF present in the 1.2 knt 5' leader of one *stuA* mRNA is required for induction-dependent positive translational regulation of StuA expression. Developmentally regulated expression of StuA is localized in specific cells of the conidiophore and in the dikaryotic tissue of the cleistothecium. Mutations in *cis*-acting elements demonstrate that the role of StuA in the sexual and asexual reproduction cycles can be separated.

Transcription of the two *medA* mRNAs is only slightly regulated in response to developmental induction. However, both mRNAs have 2 knt 5' leaders containing multiple mORFs. This observation suggests that a significant component of MedA expression may also involve regulation at the translational level. The role of MedA in the two reproductive cycles can be genetically distinguished.

[1]Mirabito, PM *et al.* (1989) Cell **57**:859-868.
[2]Miller, KY *et al.* (1991) MGG **227**:285-292
[3]Miller, KY *et al.* (1992) Genes & Dev. **6**:1770-1782

J 028 **COUPLING GENE EXPRESSION TO MORPHOGENESIS DURING SPORULATION IN *BACILLUS SUBTILIS*.**

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Synthesis of an asymmetrically positioned septum is the first recognizable morphological event during sporulation in Bacilli. It is followed by activation of sigma-F in the smaller cell and activation of sigma-E in the larger one. These two sigma factors are synthesized before septation and are held in inactive form until septation is completed. Sigma-F is inhibited by the SpoIIAB protein while sigma-E is inhibited by the presence of an amino-terminal prosequence. SpoIIAB is displaced by the action of the SpoIIAA protein which also requires the presence of another protein, the product of the *spoIIE* gene. The processing of the sigma-E prosequence depends on the *spoIIGA* gene product and also requires the presence of active sigma-F. Since SpoIIE, SpoIIAA, and SpoIIGA are all synthesized before septation and are expected to be segregated randomly in the two cell types some subtle mechanisms have to be involved for releasing the SpoIIAB inhibitory effect only in the smaller cell and for transducing this information to the processing machinery that activates sigma-E. The exact function of SpoIIE (including its intracellular localization as well as the influence of putative concentration differences between the two cells) remains to be investigated. The molecular basis for the dependency of pro-sigma-E processing on active sigma-F could be due either to the action of a gene product whose synthesis is controlled by sigma-F or to some intrinsic feature of the sigma-F polypeptide once released from its interaction with SpoIIAB. The domains of the SpoIIGA protein that are involved in the control of its processing activity have yet to be defined as well as the exact relationship between septum formation and activation of sigma-E. Identification of these "missing links" would allow a better understanding of the coupling between formation of two morphologically different cells and activation of two distinct transcription programs.

Later in sporulation transcription in the smaller cell (the forespore) depends on another sigma factor, sigma-G. Synthesis of sigma-G is confined to the forespore because transcription of *spoIIIG* (which encodes sigma-G) is initiated by sigma-F. However there is a delay between sigma-G synthesis and the induction of late forespore genes, suggesting that sigma-G is also locked in inactive form when it is synthesized. Transcription of genes controlled by sigma-G does not occur in strains that do not complete engulfment of the smaller cell by the larger one, nor in strains that achieve engulfment but contain an inactive *spoIIIA* locus. This locus encodes eight polypeptides that are all required for activation of sigma-G. Since the *spoIIIA* operon is transcribed by sigma-E its products appear to activate sigma-G from the outside of the forespore, but only after the forespore has been fully engulfed. Elucidation of the molecular basis for inhibition of sigma-G would be a first step towards understanding the role of the *spoIIIA* products and could explain how the end of engulfment might be a signal inducing a switch in forespore gene expression.

Late Abstract

REGULATION OF THE pAD1-ENCODED SEX PHEROMONE RESPONSE IN ENTEROCOCCUS FAECALIS. Don B. Clewell, Koichi Tanimoto, and Florence An. The University of Michigan, Ann Arbor, MI 48109.

pAD1 is a conjugative, 60-kb, hemolysin-bacteriocin plasmid in *Enterococcus faecalis*. It encodes a mating response to a peptide sex pheromone, cAD1, secreted by potential recipient (plasmid-free) cells. Pheromone-induced donors synthesize a microfibrillar protein (aggregation substance; Asa1) that appears on the cell surface and facilitates the formation of mating aggregates. The response is regulated by a cluster of genes designated and arranged as *-traE1-iad-traA-traC-traB-* . The product of *traE1* is a 14 kDa protein that acts as a positive regulator for all or most of the structural genes related to conjugation. The *traA* product is a protein (38 kDa) that acts as a negative regulator of *traE1* expression. The *traC* determinant encodes a surface protein (61 kDa) that binds to pheromone. *traB* encodes a product (44 kDa) that facilitates the shutdown of chromosome-determined cAD1 production in donors. *iad* encodes a peptide designated iAD1 which is secreted and represents a competitive inhibitor of cAD1; the peptide is synthesized as a 22 amino acid precursor peptide (resembling a signal peptide) that is eventually processed to the 8 amino acid-residue mature product. The segment of DNA carrying all of these determinants has been sequenced, and mutants in each have been analyzed. All of the determinants, with the exception of *traA*, are oriented right-to-left (5' to 3'). Northern blot analyses of transcription, as well as studies of transcriptional *lacZ* fusions , in the *traE1-iad* region indicate that induction by cAD1 results in a transcriptional readthrough of two apparent termination sites located between *iad* and *traE1*. The data show that once expression is initiated via transcriptional readthrough from *iad*, TraE1 can then express independently, probably as a result of activating its own promoter. This view is also supported by genetic complementation studies. DNA binding studies using TraA showed that the protein binds to the promoter of *iad*. Binding to the region between *iad* and *traE1* was not detected; however, the involvement of TraA in influencing transcription termination in this region is not ruled out, since additional factors could be involved. A model for regulation of the pheromone response has been proposed.

Polarity Determination, Subcellular Morphogenesis, and Colony Growth; Chemotaxis and Motility

J 100 PROTEOLYSIS OF A POLARLY LOCALIZED CHEMORECEPTOR RESPONDS TO TEMPORAL AND SPATIAL CONTROL DURING *CAULOBACTER* DEVELOPMENT, M.R.K. Alley, Janine R. Maddock and Lucy Shapiro, Department of Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305.

The bacterium *Caulobacter crescentus* yields two different progeny at each cell division; a chemotactically competent swarmer cell and a sessile stalked cell. The chemotaxis proteins are synthesized only in the predivisional cell and then specifically segregated to the progeny swarmer cell. Immuno-gold electron microscopy revealed that the chemoreceptors are targeted to the nascent swarmer pole of the predivisional cell and thus are partitioned to the swarmer cell upon division. When this swarmer cell differentiates into a stalked cell, the chemoreceptors are specifically degraded, coincident with the loss of the polar flagellum and pili. Thus a temporally and spatially restricted proteolytic event is a component of the differentiation process. The amino acid sequence that is required for this proteolytic event is located at the extreme C-terminus of the chemoreceptor. Derivatives of the chemoreceptor that lack this C-terminal signal are not degraded but are still retained at the cell pole. Thus demonstrating that this proteolytic event is independent of polar localization. We will also show the involvement of proteolysis in targeting the chemoreceptor to the swarmer cell upon division.

J 101 REGULATION AND LOCALIZATION OF THE FTSZ PROTEIN AND IDENTIFICATION OF THE ftsZ GENE OF Caulobacter crescentus, Yves V. Brun and Lucy Shapiro, Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305-5427.

The complex process of cell division requires the proper timing of numerous events. Genetic and biochemical studies of cell division in *E. coli* have shown that the *ftsZ* gene plays a central role in the early steps of cell division. We have initiated a study of the *ftsZ* gene of *Caulobacter crescentus*, a differentiating gram-negative bacterium that is easily synchronizable, in order to understand the temporal and spatial requirements of *ftsZ* action. The *ftsZ* gene of *C. crescentus* was cloned and sequence analysis showed a high degree of homology to *ftsZ* genes from other bacteria, including the conserved GTP binding motif. Increasing the copy number of the *ftsZ* region of the chromosome approximately two to five fold results in the production of minicells. A polyclonal antibody to *E. coli* FtsZ, which cross-reacts with a 70 kDa protein, was used to study the timing and positioning of FtsZ during the *C. crescentus* cell cycle. Indirect immunofluorescence showed that the FtsZ homologue of *C. crescentus* is localized at the division site approximately at the time of division initiation. Western blots of cells taken at different times in the cell cycle suggest that the level of FtsZ is very low in swarmer cells and increases when cell division is initiated. Furthermore, the FtsZ protein appears to be turned over after the completion of cell division. We are investigating how the temporal control of cell division genes is involved in the localized assembly of the cell division apparatus at the proper time of the cell cycle.

J 102 ASYMMETRY OF THE FLAGELLA IN *CHLAMYDOMONAS*, Susan K. Dutcher, Stephen King and Eileen O'Toole, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347

Chlamydomonas reinhardtii has two flagella that differ from one another by their position in the cell and by their behavior during phototaxis. Changes in intracellular calcium are thought to mediate a phototactic response, which turns the cell by increasing the beat frequency of one of the two flagella. The *cis* flagellum is on the side of the cell nearest to the eyespot, it is templated by the daughter basal body, and is active at calcium concentrations of 10^{-8}M and below. The *trans* flagellum is on the side opposite the eyespot, is templated by the parental basal body and is active at calcium concentrations of 10^{-8}M and above.

We are currently investigating whether there are structural differences between the *cis* and *trans* flagella in the region of the dynein arms. We have identified two *cis-trans* structural differences in mutant strains. Mutations at the *UNI1* locus in *Chlamydomonas* assemble primarily the *trans* flagellum. Using the isolated flagella from this mutant strain, longitudinal electron micrographs were analyzed by computer-aided averaging methods developed by Masteronarde *et al.* (1992). We find that a structure in the inner dynein arms region is missing in the *uni1* preparation when compared to the wild-type preparations. This suggests that there is a structure that is present in *cis* flagella and not in *trans* flagella. A second unlinked locus, *UNI2*, has been identified that also assembles primarily *trans* flagella; flagellar preparations from this strain are being investigated at present. To ask if this difference is a result of the mutation or an intrinsic difference in the *cis* and *trans* flagella, we are currently examined individual flagella from wild-type prepartions to determine if this region is missing in one-half of the flagella from wild-type prepartions.

Examination of isolated flagella from the *bop2* mutant strain, which has a motility phenotype that resembles mutant strains lacking inner arm structures, also shows a morphological difference between the *cis* and *trans* flagella. Biochemically, flagella from this strain are missing a 152,000 dalton phosphoprotein. This mutant strain fails to assemble a structure in both flagella as well as a structure that fails to assemble only in only about one-half of the flagella. When flagella from a *uni1 bop2* double mutant strain are examined, this second structure appears to be present in *cis* flagella and absent from *trans* flagella. The *BOP2* locus appears to affect the assembly of *trans* specific polypeptides.

J 103 IDENTIFICATION OF FTSA-HOMOLOGS IN PROCARYOTES, Randall Gayda and Xiaoping Shao, Department of Microbiology, Louisiana State University, Baton Rouge, Louisiana 70803.

The *ftsA* gene and its 46 kDal protein product are essential for cell division in *Escherichia coli*. The possibility that FtsA-like proteins may exist in all procaryotes was suggested by the finding of an *ftsA* gene homolog in *Bacillus subtilis* (Beall et al., J. Bacteriol. *170*: 4855, 1988). Cell extracts from twenty-eight different microorganisms were screened for reactivity to a monoclonal antibody, HW65(IgG2b), specific for *E. coli's* FtsA by Western blotting. Most species had one immuno-reactive band between 50 and 45 kDal, which included *Salmonella choleraesuis, Citrobacter freundii, Enterobacter aerogenes, Serratia marcescens, Proteus vulgaris, Rhizobium meliloti, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Clostridium sporogenes, Mycobacterium phlei, Arthrobacter crystallopoieties, Azotobacter vinelandii* and *Deinococcus radiodurans*. Several species had two or more immuno-reactive bands, which included *Vibrio cholerae, Bacillus subtilis, Streptococcus faecalis, Sarcina lutea, Sporosarcina ureae* and *Caulobacter vibrioides*. Interestingly, *Mycoplasma hyorhinis* extracts contained two FtsA immunoreactive bands, one 51 kDal and 45 kDal. Four immunoreactive FtsA bands were also detected in the Archaebacterium, *Halobacterium volcanii*. Furthermore, evidence that this protein epitope may be conserved in Eucaryotic organisms was also obtained. A cross-reactive band with the FtsA monoclonal antibody was observed in Western blots of extracts from two species of yeast, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. This evolutionary conservation of FtsA-homologs suggests that there may be a universal mechanism for cell division in microorganisms.

J 104 REGULATION OF SWARMER POLE SPECIFIC TRANSCRIPTION IN *CAULOBACTER*, James W. Gober, Marilis do Valle Marques, James A. Wingrove, Department of Chemistry and Biochemistry, University of California, Los Angeles

The transcription of three σ^{54} flagellar promoters (*flbG*, *flgK* and *flaN*) is restricted to the swarmer pole of the predivisional cell of *Caulobacter crescentus*. The assembly of the flagellum at the swarmer pole, is one developmental event that may influence transcription of these genes. For example, early flagellar structural gene products (Class I genes) are required for *flbG* transcription. To identify genes that link flagellar assembly to *flbG* transcription, we have isolated extragenic mutations that no longer require Class I flagellar gene products to activate *flbG* transcription. These by-pass mutations apparently uncouple *flbG* transcriptional activation from flagellum assembly events. We are now using these mutant strains to identify mutations in the genes that encode bona fide trans-acting factors. Thus far, we have found that these mutations are unable suppress the transcriptional requirement for only one gene, *flbD*. Sequence analysis has demonstrated that FlbD protein is homologous to transcriptional activators of σ^{54} promoters (Ramakrishnan and Newton, 1990). We have now purified the FlbD protein and find that it binds specifically to the upstream enhancers of *flbG* and *flgK*. In addition, FlbD will not bind to mutant enhancer sequences that cannot activate transcription in vivo. These data indicate that FlbD may be the transcriptional activator of these promoters. To investigate the role of FlbD in compartment-specific transcription, we have tagged the carboxyl terminus of FlbD protein with the eight amino acid, M2 epitope. We then assayed the intracellular location of epitope-tagged FlbD using immunofluorescence microscopy with anti-M2 antibody. Both predivisional cell compartments contained equal amounts of epitope-tagged FlbD. These results suggest that spatially restricted transcription of flagellar genes is not due to the specific localization of FlbD. Therefore, if FlbD is responsible for the compartmentalized expression of flagellar genes, its activity must be restricted to the swarmer cell compartment. We are currently determining what factors regulate the activity of FlbD in a compartment-specific manner. In this regard, we have found that pure FlbD can be phosphorylated using cell free extracts. We are currently identifying the cellular cues that influence FlbD phosphorylation.

J 105 GENETIC AND MOLECULAR ANALYSIS OF SEPTATION IN *ASPERGILLUS NIDULANS*, Steven D. Harris and John E. Hamer, Dept. of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN 47906

Septum formation is believed to play a critical role during fungal morphogenesis. We have undertaken a combined genetic and molecular analysis of septum formation in *Aspergillus nidulans* to determine how septa are assembled and to elucidate the mechanisms that spatially and temporally coordinate this assembly with other cell cycle events.

Vegetative hyphae of *A. nidulans* are composed of septal compartments that are multinucleate. In germinating conidia, septa do not form until at least three rounds of nuclear division have occurred. The first septum always forms in the basal region of the germ tube. Measurements of cell size suggest that germlings make their first septum within a particular size (or volume) range. However, using hydroxyurea to inhibit the nuclear division cycle, we have observed that irregardless of size (or volume), germlings must at least be in the process of completing their third round of nuclear division before they are able to make a septum.

Inhibitors and temperature-sensitive mutants that block at particular stages of the nuclear division cycle were utilized to determine which events are necessary for septation to occur. Germlings treated with benomyl during the second round of nuclear division fail to make a septum while those treated later are generally able to do so. The temperature-sensitive *bimB* and *bimC* mutants of *A. nidulans* undergo multiple rounds of replication without completing a single mitosis. Each of these mutants is unable to make a septum when incubated at restrictive temperature. The temperature-sensitive *nudA* and *nudC* mutants exhibit a normal nuclear division cycle but are defective in nuclear migration. Each of these mutants is able to make septa when incubated at restrictive temperature. These results suggest that during the third round of nuclear division, events associated with mitosis are critical for septum formation.

Four *A. nidulans* temperature-sensitive mutants exhibiting defects in septation but not in nuclear division have been previously reported (Morris, R., 1976, *Genet. Res., Camb.*, 26:237). These mutants grow normally at 28°, but fail to make septa and eventually cease growth at 42°. Results from a series of temperature shift experiments suggest that the defect in the *sepA* mutant occurs during the process of assembling the septum. In contrast, similar experiments with the *sepB* mutant indicate that it is defective in the spatial (and perhaps temporal) regulation of septum formation. We have mapped each of these mutations and have initiated attempts to clone both genes.

J 106 MICROTUBULES AND CELL POLARITY DURING THE CELL CYCLE OF *USTILAGO MAYDIS*, Charles W. Jacobs, S.J. Mattichak, and J. F. Knowles, Department of Biology, Albion College, Albion MI 49224

Sporidia of the fungus *Ustilago maydis* FB2-47 *a2 b2 ade-* reproduce by forming one bud at the pole of the mother cell each cell cycle. Time-lapse microscopy revealed that the first bud from a new cell usually emerged distal to the point of attachment to the mother cell. Mother cells tended to alternate the pole at which the bud formed in subsequent cell cycles. Log-phase cells were observed with multiple buds that had emerged during successive cycles from different bud sites. Moreover, transmission and scanning electron microscopy showed evidence of repeated use of the same bud site. We conclude that *U. maydis* uses a limited number of potential bud sites, but uses some of them repeatedly.

Based on counts of 1000 DAPI-stained, log-phase cells, 54% of the cells were unbudded and uninucleate, 38% were budded with the nucleus in the mother cell, 2% budded with the nucleus in the neck, 1% had a dividing nucleus, and 6% were budded with one nucleus in the mother cell and one in the bud.

Immunofluorescence microscopy revealed that there are two distinct categories of microtubules in *Ustilago*: cytoplasmic microtubules that extend roughly between the poles of the cell and do not appear to emanate from spindle pole bodies, and spindle microtubules. The former are present during most of the cell cycle, and extend into the developing bud, but cannot be seen during nuclear division. The latter were detected only during nuclear division. In this respect, the microtubule cycle of *Ustilago* resembles that of most animals and higher plants. It appears that the transition between the cytoplasmic network and spindle microtubules occurs as the nucleus transists the neck between the mother cell and bud.

We further investigated the inhibition of cell division by Benomyl. Whereas the control cultures displayed log-phase growth and normal budding patterns similar to those described above, Benomyl (4 µg/ml) inhibited growth, and caused cell cycle arrest. In addition, Benomyl caused changes in cell morphology, including swelling of mother cells, multiple budding, and malformation of buds.

J 107 THE *E. coli surA* GENE PRODUCT, ESSENTIAL FOR SURVIVAL DURING STATIONARY PHASE, AFFECTS CELL WALL STRUCTURE, Sara Lazar, Marta Almirón and Roberto Kolter, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

Upon the onset of starvation, microorganisms generally develop into forms that exhibit increased resistance to a variety of environmental challenges. This general increase in resistance is accompanied by marked changes in cell morphology. *E. coli*, normally rod shaped during growth, differentiates into a spherically shaped cell when starved. It is therefore not surprising that the overall structure of the cell envelope is different in growing and non-growing cells.

Using a screen developed to detect mutations that affect cellular survival during stationary phase, we identified an insertion in a gene designated *surA*. This insertion results in a strain that completely loses viability after the cessation of growth. The mutant cells have normal morphology during growth but become misshapen and eventually lyse during prolonged incubation in stationary phase. The mutant cells also show increased sensitivity to β–lactam antibiotics and decreased ability to withstand pH extremes, suggestive of cell envelope defects. HPLC analysis of the chemical structure of the mutant cell wall indicates that the *surA* gene product is somehow involved in determination of cell wall structure. The nucleotide sequence of *surA* predicts that the product is a periplasmic protein, but contains no amino acid sequence similarities to known proteins.

J 108 THE CHEMORECEPTORS AND CHEMOTAXIS SIGNAL TRANSDUCTION PROTEINS ARE CLUSTERED AT THE POLE OF THE *E. COLI* CELL. Janine Maddock and Lucy Shapiro. Department of Developmental Biology, Stanford University, Stanford, Ca. 94305-5427.

We are using both immuno-electron microscopy and immunofluorescence to examine the *in vivo* organization of chemotaxis proteins in the bacterial cell. In *Caulobacter crescentus* the chemoreceptor McpA is synthesized in the predivisional cell and localized to the flagellated pole. When the *E. coli* Tsr is expressed in *Caulobacter*, it is localized to the cell poles.

The *E. coli* chemotaxis proteins, MCP, CheA and CheW have been shown to form a ternary complex *in vitro* (Gegner *et al*, Cell 1992) When we examined the intracellular positioning of these proteins in *E. coli*, we found that they are predominantly distributed in clusters associated with the inner membrane at the poles of the cells. Using anti-MCP antibodies on a wild-type strain we have shown that 80% of the membrane associated gold particles are at the cell poles. The polar clustering of the MCP is dependent upon the presence of both CheA and CheW. The distribution of both CheA and CheW parallels that of the MCPs. In the absense of chemoreceptors, CheA and CheW become cytoplasmic. Thus, the polar positioning, as well as the clustering of CheA and CheW is absolutely dependent upon the presence of the MCPs. The positioning of CheA and CheW are partially dependent upon each other. None of the proteins examined require the CheY or CheZ signal transduction proteins for polar localization.

We are defining the sites on the chemoreceptor that are essential for polar localization and we are analyzing the biological relevance of polar clustering.

J 109 DIFFERENTIAL EFFECTS OF EXPRESSION OF THE TWO *FTSZ* CELL DIVISION LOCI IN *RHIZOBIUM MELILOTI*. William Margolin and Sharon R. Long, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

The FtsZ protein is essential for the initiation of division septum formation in *E. coli* and *B. subtilis*, and is a target of several cell division inhibitors. We are studying cell division arrest during the differentiation of *R. meliloti* bacteroids in alfalfa nodules, and have focused on *ftsZ* as it is a likely target for division inhibition. *R. meliloti* expresses two novel FtsZ proteins of 63 kDa (Z1) and 36 kDa (Z2) that have extensive similarity to the 40 kDa *E. coli* and *B. subtilis* FtsZs; however, Z1 contains a large insertion, while Z2 is missing the C-terminal residues found in Z1 and *E. coli* and *B. subtilis* FtsZ. The *ftsZ1* and *ftsZ2* loci are about 100 kb apart on the main *R. meliloti* chromosome, and not on either of the two symbiotic megaplasmids. Although an insertion mutation in *ftsZ2* had no effect on cell viability, no viable insertions have been isolated in *ftsZ1*, suggesting that it is essential in free-living bacteria. Overproduction of either Z1 or Z2 in *E. coli* inhibited *E. coli* cell division, as is observed with overproduction of *E. coli* FtsZ. Moderate overproduction of Z2 in *E. coli* resulted in cells with severe kinks, twists, and large polar blebs. Overproduction of Z1 and Z2 in *R. meliloti* resulted in different morphological abnormalities: Z1 overexpression yielded cells with swollen midpoints, while Z2 overexpression resulted in cells with swollen, multiple branches. In neither case did we observe cell filamentation as seen in *E. coli*. These results suggest that the mechanism of *R. meliloti* division may be under different controls than that of *E. coli*. Using *ftsZ*-GUS fusions, we are currently studying how these genes are regulated in free-living bacteria and in differentiating bacteroids.

J 110 CELL/COLONY MORPHLOGY (CCM) TYPE SWITCHING IN *SACCHAROMYCES CEREVISIAE*: A MODEL FOR CELLULAR DIFFERENTIATION. John H. McCusker and Ronald W. Davis. Dept. of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

A novel cell type (distinct from both MAT and pseudohyphal cell types) switching system has been found in *S. cerevisiae*. Cell/colony morphology (CCM) type switching has been found in clinical *S. cerevisiae* strains. CCM switching produces multiple morphologically and physiologically different colony types with (in some cases) changes in cellular morphology. In some strains, CCM switching resembles the white/opaque transition of *C. albicans*. CCM cell types differ physiologically with respect to maximum growth temperature, sugar fermentation and protease production i.e. a CCM switch affects many diverse properties and is extremely pleiotropic. CCM switching is dominant in crosses with lab strains, occurs at a single locus and is *rad52* independent. In addition to CCM switching, the clinical *S. cerevisiae* isolates are capable of growing at 42° a temperature which completely blocks the growth of lab (and other) strains. The ability to grow at very high temperatures is dominant in crosses with lab strains and is polygenic with complex additive and epistatic effects i.e. a quantitative trait. Maximum growth temperature is affected by CCM switching. Many of the clinical isolates appear to be able to secrete protease as judged by their ability to liquefy gelatin and/or to utilize casein as a sole nitrogen source. Extracellular protease production is thought to be a virulence trait in pathogenic fungi. Gelatin liquefaction and casein utilization is affected by CCM switching. Colony morphology switching has been postulated to be important in *C. albicans* pathogenesis. CCM switching serves as a model for cellular differentiation which appears to be relevent to fungal pathogenesis.

J 111 HEAT SHOCK PROTEINS IN HYPHAL BRANCHING AND SECRETION IN STEROID HORMONE INDUCED FUNGAL DEVELOPMENT. Julie C. Silver, Shelley A. Brunt, Garyfallia Kyriakopoulou, Monica Borkar and Vigen Nazarian-Armavil. Department of Microbiology, Division of Life Sciences, University of Toronto, Scarborough Campus (JCS, SAB, GK, and MB) and Pioneer Hi-Bred Ltd. (VNA), Toronto, Ontario, Canada M1C 1A4.

In the filamentous oomycete fungus Achlya ambisexualis, the differentiation on vegetative hyphae of the male mating type, of gamete bearing structures, is induced by the Achlya steroid mating hormone, antheridiol. Among the several metabolically labeled intracellular proteins whose synthesis or accumulation is altered by hormone-treatment, are steroid-induced 85kDa and 68-78kDa proteins. The 85kDa protein was previously shown to be the Achlya heat shock protein hsp85 [Brunt et al., 1990, Brunt and Silver 1991] a component of the putative Achlya steroid hormone (antheridiol) receptor. It was of interest to determine if the antheridiol-induced "70kDa" proteins were hsp70-family heat shock proteins and if hormone treatment induced changes in the expression of hsp70 transcripts. Three different Achlya hsp70 genomic sequences were cloned and used to investigate these questions. The three hsp70 sequences recognized three different mycelial transcript populations, one of which was regulated also by decreased glucose, tunicamycin and calcium ionophore A23187. Of note, all three hsp70 transcript populations were found to be regulated by antheridiol. The hormone-induced changes in hsp85 and hsp70 transcript levels were temporally correlated with the onset of massive lateral hyphal branching and alterations in the pattern of secreted N-linked glycoproteins which occur in hormone-treated mycelia. These observations have implications for the role of heat shock proteins in hyphal branching, secretion and development in filamentous fungi and perhaps other cell types.

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Sex Determination and Mating Factors; Cell Cycle Controls in Cellular Differentiation

J 200 CHARACTERIZATION OF A YEAST MEIOTIC ACTIVATOR, Mary J. Clancy, Stephen R. Peterson, Xuebo Chen and Jyoti C. Shah. Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148 USA

Sporulation in the yeast, *Saccharomyces cerevisiae*, is a cell type specific response to nutrient limitation. Of the three cell types, the *MATa/MAT α* diploid is the only one that can sporulate; cells that express only one of the mating type alleles cannot do so. *IME4* (inducer of meiosis) is one of several essential activators that is expressed at high levels only in sporulating cells. Normally, *IME4* activity is essential for expression of the other meiotic activators (*IME1* and *IME2*) and for sporulation. However, mutations that bypass the requirement for both *MAT* alleles (*rme1* and *RES1-1*) also obviate the requirement for *IME4* in the induction of *IME1*.

The sequence of the *IME4* region reveals a single open reading frame that could encode a protein 600 amino acids in length. The predicted sequence includes a glutamine/asparagine-rich C-terminus and an adjacent basic region, but no DNA binding domain. To test whether *IME4* could provide an activation domain to DNA-bound proteins, we fused most of the *IME4* coding region to the DNA binding domain of *E. coli lexA*. These constructs did not activate transcription from a *lexA* operator-*CYC1-lacZ* fusion reporter in yeast.

Strains that contain *LEU2* or *TRP1* inserted within the coding region of *IME4* are sporulation defective, whereas insertions that are just 3' to the coding region promote *IME4* expression and sporulation in inappropriate cell types. *IME4* is also induced to unusually high levels in starved *MATa/MAT α* diploids. Thus, the 3' insertions may alter the stability of the *IME4* mRNA. Analysis of the *IME4* regions reveals an adjacent transcript originating on the opposite strand that is expressed highly in haploids but not in *MATa/MAT α* diploids. This transcript overlaps the *IME4* region over much of its length, but does not contain any open reading frames.

J 201 MOLECULAR ANALYSIS OF A *CIS*-ACTING, ORIENTATION-DEPENDENT POSITIVE CONTROL SYSTEM OF PHEROMONE-INDUCIBLE CONJUGATION FUNCTIONS IN *ENTEROCOCCUS FAECALIS*, Gary M. Dunny, Jungwon W. Chung and Barbara A. Bensing, University of Minnesota, St. Paul, MN 55108.

The *prgB* gene encodes the surface protein Asc10, which mediates cell aggregation resulting in high frequency conjugative transfer of the pheromone-inducible tetracycline resistance plasmid pCF10 in *Enterococcus faecalis*. Previous Tn*5* insertional mutagenesis and sequencing analysis of a 12 kb region of pCF10 indicated that the upstream region encoding *prgR* and *S* is required to activate the expression of *prgB*. Northern blot hybridization and primer extension analysis indicated that *prgB* is transcribed monocistronically and inducibly by the addition of pheromone. The distance (3-4 kb) between these regulatory genes and *prgB* suggested that the activation might function *in trans*. However, numerous complementation studies showed that the activation of *prgB* occurred only when the regulatory region and target genes were cloned *in cis*. Interestingly, this activation was dependent on the two regions being cloned in the same relative orientation in which they exist on wild-type pCF10, although they could be separated as much as 12 kb. Further deletion and transcriptional analysis of the regulatory region suggested that an additional upstream region encoding a gene called *prgQ* is also required for *prgB* activation and that the functional products of some of the genes in the regulatory region might be RNA molecules rather than proteins. The model which best fits the currently available data is that one or more regulatory molecules may bind to an upstream regulatory sequence and track along the DNA in a specific direction to reach a target site.

J 202 MARKER EXCLUSION MEDIATED BY AN INTRON-ENDONUCLEASE, Heidi Goodrich-Blair and David A. Shub, Biological Sciences and Center for Molecular Genetics, University at Albany, SUNY, Albany, NY 12222

The virulent *Bacillus subtilis* bacteriophage SPO1 and SP82 belong to a closely related family that contain hydroxymethyluracil (HMU) in place of thymine in their DNA. A previous report indicated that when these two phage coinfect a single cell, SP82 genetic markers are more likely to be carried by the progeny than those of SPO1. This exclusion phenomenon occurs over ten kilobases of DNA, surrounding the DNA polymerase gene [1]. We have found that this exclusion is dependent on the expression, by SP82, of a site- and strand-specific endonuclease, I-Hmu II. The gene for the endonuclease lies entirely within a self-splicing group I intron interrupting the SP82 DNA polymerase gene. The target site of I-Hmu II is within the SPO1 DNA polymerase gene, 54 bp from the 3' splice-site of the intron. We hypothesize that this cleavage initiates unidirectional gene conversion in the region radiating from the cut site. Several examples of such a process exist: yeast HO endonuclease cleavage causing mating type switching and intron-encoded endonuclease cleavage causing "homing" of group I introns. In each case the endonucleases introduce a specific double strand cut that is repaired and "converted" using cellular pathways of recombination and repair. In homing, the intron-endonucleases specifically recognize and cleave intron-less alleles. Repair of the cleavage using the intron-plus allele as a template results in two intron-plus copies of the gene. This process ensures the propagation of both the endonuclease and the intron in which it resides. Unlike these intron-endonucleases, I-Hmu II introduces a single strand cut, perhaps reflecting a difference in *B. subtilis* recombination pathways. Also, it is able to cleave intron-plus as well as intron-less DNA of a heterologous genome. It seems, therefore, that the function of I-Hmu II has expanded beyond intron homing to include the propagation of other genes of its host.

1. Stewart, C. and Franck, M., *J. Virology* **38** 1081-1083 (1981).

J 203 DEVELOPMENTAL CONTROL OF DNA REPLICATION IN C. crescentus, Gregory T. Marczynski, Krista Lentine, and Lucy Shapiro, Dept. of Developmental Biology, Stanford University Medical Center, Stanford, CA 94305.

C. crescentus cell division is asymmetric and yields swarmer and stalked cell progeny. Only the stalked cell initiates chromosomal replication; the swarmer cell must differentiate into a stalked cell in order to replicate its chromosome and divide. To determine the developmental control of replication initiation, we localized, cloned, and sequenced the C. crescentus replication origin. A plasmid that employs only the C. crescentus origin of DNA replication initiates replication at the same time in the cell cycle as the bona fide chromosome, suggesting that cis-acting control elements are linked to the origin. In vitro mutagenesis of the origin revealed that it contains essential sequences that are common to other bacterial origins, such as DnaA boxes and an A+T-rich region. This origin also requires unique motifs for replication function: Six (AAGCCCGG) motifs and five (GTTAA-N7-TTAA) motifs. Another unique feature of this origin is its overlap with a complex promoter region of a heme biosynthetic gene (hemE). We will present a detailed characterization of this unusual promoter, and we propose a model that links transcription to replication within the C. crescentus origin of replication.

J 204 ANALYSIS OF THE GENETIC TRANSFER SYSTEM OF THE HALOPHILIC ARCHAEBACTERIUM *Haloferax volcanii*, Moshe Mevarech, Ronen Tchelet and Ron Ortenberg, Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv 69978, Israel

The genetic transfer system in the extremely halophilic archaebacterium *Haloferax volcanii* is the only archaebacterial mating system known. This system is characterized by: i) being dependent on contact between two living parental cells; ii) being bidirectional, namely, it is impossible to distinguish between donor cells and recipient cells. It was previously shown [Science **245**: 1387-1389 (1989)] that whereas chromosomal markers are transferred in the process of mating, two naturally occurring plasmids of *H. volcanii* (pHV2 and pHV11) are not transferred in the process. This observation was interpreted as an indication that the genetic transfer is not the result of fusing cells.

Recently we have shown the unlike pHV2 and pHV11, the halobacterial shuttle plasmids pWL102 and pMDS1 constructed by combining the origins of replication of pHV2 and pHK2 (another resident plasmid of halobacteria) to *E. coli* plasmid origins and to halobacterial selectable markers, can be transferred. These shuttle vectors were found, however, to be relatively unstable and are lost when no selection for their corresponding markers is imposed. We have found that this instability is due in part to the elimination of parts of the original plasmids in the process of construction of the shuttle vectors. These missing parts contain several open reading frames that are probably responsible for the maintenance of the plasmids in the cell. We are trying now to determine whether there is a correlation between the maintenance functions and the ability (or rather inability) to transfer plasmids in the mating process.

Using transferable and selectable shuttle vectors we could demonstrate interspecies genetic transfer. After crossing *H. volcanii* carrying pWL102 that confers resistance to mevinolin with *H. mediterranei* carrying pMDS1 that confers resistance to novobiocin and selecting for colonies resistant to mevinolin and novobiocin, colonies of *H. mediterranei* carrying the two plasmids are obtained.

The aims of the present work are to find what are the cellular components involved in the recognition of the cells, the genes involved in the transfer process and the *cis* elements in the chromosome which are required for chromosome transfer.

J 205 NOVEL PROMOTER ARCHITECTURE OF THE EARLY *Caulobacter* FLAGELLAR GENES *fliLM* AND *fliQR* . Craig Stephens, Wei Yun Zhuang, and Lucy Shapiro. Dept. of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305.

A subset of flagellar genes, including the *fliLM* and *fliQR* operons, have been identified which occupy the highest level of the flagellar regulatory heirarchy. Expression of *fliLM* and *fliQR* is temporally regulated, and their transcription is inhibited if DNA replication is interrupted. The two operons have a highly conserved promoter region (shown below). This sequence does not resemble known bacterial promoters, suggesting a role for a novel sigma factor.

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                 **      ** *  *        *
fliLM (-39) AAACACATCGTTAACCATGCTTCGCGCATGAG (-8)
            ||| |   |||||||||  ||||  |||
fliQR (-37) TAACGCCCTGTTAACCATATTTCGTCCATCTT (-6)
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In vitro mutagenesis has defined the nucleotide sequence requirements for transcription and temporal regulation. The activities of both promoters were severely reduced by deletion to the -25 region, by linker insertion at this site, and by multiple base changes in the conserved sequences around -35 and -12. The effects of over 30 single base substitutions in the *fliL* promoter have been characterized by fusion to *lacZ*, and additional mutations are currently being analyzed in the *fliQ* promoter. Positions at which substitutions reduce activity by greater than 50% are indicated by asterisks; each of these bases is conserved in the two promoters. All substitutions outside of the conserved regions have less than a 2-fold effect on activity. With one possible exception, the mutants tested thus far exhibit normal timing of transcription during the cell cycle.

The apparent dependence of transcription on three distinct sequence motifs (-38 to -36, -29 to -22, and -14 to -12) is unusual, as bacterial promoters tend to be bipartite. Our working model is that the -12 and -36 elements may represent RNAP contact sites, while the central element constitutes a binding site for a separate transcriptional regulator. We are currently identifying RNAP holoenzyme and other factors that interact with these promoters.

J 206 GENETIC ANALYSIS OF THE FUNCTION OF Z AND Y HOMEODOMAINS IN Z/Y REGULATION OF SCHIZOPHYLLUM DEVELOPMENT R.C. Ullrich[1], Y. Luo[2] and C.P. Novotny[2], Departments of Botany[1], and Microbiology and Molecular Genetics[2], University of Vermont, Burlington, VT 05405

The Aα mating locus is one of four loci that regulate sexual development in the fungus Schizophyllum commune. The locus consists of two dissimilar multiallelic genes, Y and Z. There are nine alternative forms of Aα in nature. The Z polypeptides encoded by different alleles are 42% identical. The Y polypeptides exhibit 49-54% identity. The deduced Z and Y polypeptides possess homeodomain motifs that may enable them to bind DNA and thereby regulate the expression of developmental genes. Transformation experiments show that the A developmental pathway is activated when Z from one Aα interacts with Y from a different Aα.

We made deletions and point mutations in the homeodomain regions of Z and Y and tested the mutagenized genes in transformation for their ability to activate the A developmental pathway. The results show that the homeodomain of Y is essential for Y/Z activation of development, whereas the homeodomain of Z is not.

Multicellular Development; Spore Formation

J 300 CLONING AND CHARACTERIZATION OF A GENE REQUIRED FOR THE ASSEMBLY OF THE BACILLUS SUBTILIS SPORE COAT. Bernard Beall[1], Adam Driks[2], and Charles P. Moran Jr.[1] [1]Department of Microbiology and Immunology, Emory University, Atlanta, GA. 30322, [2]The Biological Laboratories, Harvard University, Cambridge, MA. 02136.

During endospore formation in Bacillus subtilus approximately a dozen proteins are synthesized and assembled around the spore to form a protective coat. Little is known about the assembly process but several of the genes encoding these coat proteins are expressed in the mother cell compartment, where the proteins accumulate on the outer side of the forespore membrane. Transcription of these genes is directed by the mother cell specific sigma factor, σ^K, during the later stages of endospore development. σ^E may direct expression of the genes that encode proteins that function in the earliest stages of coat assembly. By screening for σ^E-dependant promoters we cloned a gene, designated spoVID, required for assembly of a normal spore coat. Expression of spoVID was initiated at about the second hour of sporulation and continued throughout development from a σ^E-dependent promoter. The spoVID gene was located on the Bacillus subtilis genetic map just downstream of the previously characterized hemAXCDBL operon, and is predicted to encode an extremely acidic protein of 575 residues. Insertional inactivation of spoVID, produced a strain that formed refractile spores that were resistant to heat and to chloroform, but sensitive to lysozyme. Electron microscopy of these cells revealed normal development up to about the third hour of sporulation. However, during the later stages of development the spore coat proteins appeared to be loosely associated with the forespores rather than correctly assembled into the spore coat. Many forespores appeared to have abberant spore coats with multiple alternating inner and outer layers.

J 301 ISOLATION AND ANALYSIS OF CLOCK-CONTROLLED GENES IN *Neurospora crassa*

Deborah Bell-Pedersen, Jay C. Dunlap, and Jennifer J. Loros
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One approach to understanding the mechanism by which the clock controls cellular metabolism is through the identification and analysis of genes that are expressed in a circadianly regulated fashion. To this end, rhythmically expressed genes were targeted for isolation in *Neurospora crassa* using subtractive hybridization. These studies led to the discovery of two transcriptionally regulated "morning"-specific genes, *ccg*-1 and *ccg*-2[1,2]. The *ccg*-2 gene has been analyzed and was found to be allelic to one of the first developmental loci identified in *N. crassa*, *eas*, and to encode a polypeptide required for the formation of the outer conidial rodlet layer. The rodlet layer contributes to the overall hydrophobicity of the spore and thus, plays an important role in spore dispersal. These data demonstrate a role for circadian regulation in the development of filamentous fungi, and their selective adaptation to the environment in which they grow. We are now using molecular and biochemical techniques to identify both the *cis* - and *trans* -acting elements involved in clock regulation of *ccg*-2.

In addition, we have extended the search for clock-regulated genes and have generated time-specific cDNA libraries representing morning (CT0), midday (CT6), evening (CT12) and midnight (CT18). Initial use of these libraries in a differential screen has identified several new clock-controlled genes, most of which are morning specific. We are currently characterizing these genes and gene products in order to determine each gene's role in circadian output and/or clock function.

[1]Dunlap, J.C. 1990 Trends Genet 6:159-165.
[2] Loros, J.J., and Dunlap, J.C. 1991 Mol. Cell. Biol. 11: 558-563.

J 302 Isolation and structural characterization of a putative spore pigment precursor in *Aspergillus parasiticus*.

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Asexual reproduction in several species of the ascomycetous fungus *Aspergillus* (eg. *parasiticus, flavus,* and *nidulans*) culminates in the production of green pigmented spores. These spores are more resistant to exposure to UV light and other environmental stresses than hyaline (pigment-free) spores. For example, in *A. parasiticus,* both white and orange mutants fail to develop the green pigment and are 100 and 7 fold more sensitive, respectively, to UV induced damage then wild-type strains (unpublished data). The normal, spore pigment maturation process proceeds from white or colorless to orange and finally to a mature green over a five or six day period. The native green pigment is a high molecular weight material that is refractory to direct structural analysis (Clutterbuck, personal communication and personal observation). It has been proposed that the orange material that accumulates in strains defective in p-diphenol oxidase (laccase, E.C. 1.10.3.2) is an intermediate in the pigment biosynthetic pathway.

The characterization of putative pigment precursors will aid in understanding the final pigment structure and its role in protecting the spore. To this end, we have examined at the biochemical level, a critical step in the biosynthesis of the green conidial pigment of *Aspergillus parasiticus*. This was accomplished by isolating and characterizing the orange pigment intermediates that accumulate in mutants defective in the pigmentation process. The complete structural elucidation of the spore-specific primary orange pigment is presented.

J 303 MUTATIONS INDUCING ECTOPIC SPORULATION IN *STREPTOMYCES COELICOLOR* A3(2), Mark J. Buttner, Kitty A. Plaskitt and Cinzia G. Lewis, School of Biological Sciences, University of East Anglia, University Plain, Norwich NR4 7TJ, UK and John Innes Institute, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

Members of the genus *Streptomyces* are Gram-positive soil bacteria with a mycelial growth habit. Dispersal of streptomycetes growing on solid substrates is achieved by the metamorphosis of specialised aerial hyphae into chains of uninucleate spores. Sporulation is normally confined entirely to the aerial hyphae and the normal developmental fate of the subterranean vegetative hyphae is death; during sporulation the vegetative mycelium undergoes extensive lysis.

We have discovered two strains of *S. coelicolor* A3(2), J1668 and J1987, that sporulate ectopically in the substrate hyphae (the Esp phenotype). Examination of these and related strains showed that this phenotype is caused by the deletion of DNA that lies close to, but is distinct from, the glucose kinase gene (*glk*), in the "11 o'clock" region of the chromosome. The size of the deletion in J1668 was estimated by pulsed field gel electrophoresis to be less than 10 kb, making it feasible to clone the *esp* locus by genomic walking from *glk*.

J 304 ROLE OF THE SpoIIIE PROTEIN IN THE REGULATION OF σ^F ACTIVITY DURING SPORULATION IN *Bacillus subtilis*, Jeffery Errington, Lingjuan Wu and Rudolf Allmansberger, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

The establishment of prespore-specific gene expression during sporulation in *B. subtilis* is determined by the controlled release of σ^F activity in this compartment after spore septum formation (reviewed by Losick & Stragier, 1992, *Nature* **355**, 601). Several genes encoding products that play essential roles in the regulation of σ^F activity are known: *spoIIAA*, *spoIIAB*, and *spoIIE*. The possible role of a fourth gene, *spoIIIE*, in the control of σ^F activity (Foulger & Errington, 1989, *Mol. Microbiol.* **3**, 1247) has been complicated by the finding that σ^F-dependent reporter genes placed near the origin of replication (e.g. at the *amyE* locus) become independent of *spoIIIE* (Sun *et al.*, 1991, *J. Bacteriol.* **173**, 7867). We have now found that null mutations in *spoIIIE* have the opposite effect to the classical *spoIIIE* mutations, i.e. they cause overexpression of σ^F-dependent genes. From the DNA sequences of more than 10 *spoIIIE* mutations we deduce that a C-terminal domain is involved specifically in the stimulation of σ^F activity in the prespore. A distinct function, which is independent of the extreme C-terminal part of the protein, exerts an inhibitory effect on σ^F activity from Stage II onwards.

We have partially purified fragments of the SpoIIIE protein expressed in *E. coli*, and used these to raise polyclonal antisera. Immunoblot experiments show that the SpoIIIE protein is present throughout growth and sporulation, in accordance with previous studies of *spoIIIE* transcriptional regulation (Foulger & Errington, 1989, *Mol. Microbiol.* **3**, 1247). Remarkably, the protein undergoes a specific proteolytic cleavage during sporulation. This cleavage depends on σ^E, and thus probably occurs specifically in the mother cell. Taken together, our results suggest that SpoIIIE plays an important role in the spatial regulation of σ^F activity, probably both stimulating this activity in the prespore and inhibiting it in the mother cell. Possible mechanisms for the action of SpoIIIE in the spatial regulation of σ^F activity will be discussed.

J 305 CELL DENSITY SENSING IN *DICTYOSTELIUM*, Richard H. Gomer, Ita S. Yuen, Renu Jain and Robin Ammann, Howard Hughes Medical Institute, Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251-1892

Very little is known about how the size and density of a tissue are sensed by individual cells. During development of the simple eukaryote *Dictyostelium discoideum*, the expression of several genes is regulated in part by cell density. For instance, prespore genes can be expressed by high-density starved cells but not by low-density starved cells. The density sensing is mediated by a secreted factor: low-density cells will express prespore genes when exposed to starvation buffer previously conditioned by a high density of starved cells (conditioned medium). Fractionation of conditioned medium on Sephadex G-50 shows two size classes of density sensing factor activity (also known as Conditioned Medium Factor or **CMF**). One can be purified to an 80 kD glycoprotein while the other is a set of 0.5-6 kD polypeptide breakdown products of the 80 kD CMF. The 80 kD CMF cDNA has been cloned, and the predicted amino acid sequence of CMF shows little similarity to any known protein. Northern blots show the presence of CMF mRNA in vegetative and early developing cells and undetectable levels during aggregation and later development. In the vegetative cells, CMF is sequestered in a membrane fraction but is not secreted. Immunofluorescence with antibodies directed against bacterially synthesized CMF show a combination of diffuse and punctate staining in vegetative cells; Western blots indicate that starving but not vegetative cells secrete CMF. Antisense transformation with portions of the CMF cDNA gives rise to transformants that do not secrete CMF or contain the sequestered vegetative CMF. The aggregation of these transformants is greatly delayed; however exposure to purified CMF restores a normal development pattern. This suggests that CMF might synchronize the onset of development in *Dictyostelium* by triggering aggregation when a majority of the cells are ready to aggregate, as signalled by CMF secretion. The absence of CMF appears to prevent aggregation by blocking signal transduction from the chemotactic cAMP receptor. Several hours after the completion of aggregation, CMF is converted to a set of breakdown polypeptides that have higher specific activities, which might then allow cells which did not have a sufficient number of CMF-secreting cells nearby to differentiate. Bacterially synthesized CMF has CMF activity, and a bacterially synthesized fragment corresponding to a region near the N terminus has even higher activity. Diffusion calculations using the measured secretion rate (12 molecules/cell/minute) and sensitivity of cells to the 80 kD CMF (0.3 ng/ml) suggest that a cell will not differentiate if it does not have CMF- secreting cells in its vicinity, but will differentiate in the presence of a high density of other starved cells. Interestingly, for aggregates of fewer than roughly 200 cells, the calculations indicate that the CMF concentration fails to rise to 0.3 ng/ml.

J 306 CONTROL OF EXPRESSION OF THE GENES ENCONDING SUBUNITS OF cAMP-DEPENDENT PROTEIN KINASE DURING DIFFERENTIATION IN B.EMERSONII, Suely L. Gomes, Julio Cesar F. de Oliveira, Ana Claudia C. Borges and Marilis V. Marques, Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, S.P. 01498, Brasil.

The aquatic fungus Blastocladiella emersonii constitutes an interesting system for studying the regulation of expression of regulatory (R) and catalytic (C) subunits of cAMP-dependent protein kinase (PKA). Blastocladiella cells contain a single PKA with characteristics very similar to type II kinases of mammalian tissues. cAMP-dependent protein kinase activity as well as cAMP-binding activity change drastically during Blastocladiella development. Both activities are low in vegetative cells, rising sharply during sporulation to reach maximum levels at the zoospore stage. During germination both activities decrease to the original basal levels. Western blot analyses, using anti-C and anti-R antisera, have shown that the concentration of C and R subunits, throughout the life cycle of the fungus, closely parallel kinase and cAMP-binding activities, respectively. Furthermore, the levels of C and R mRNAs, at the different stages of development, present the same pattern of variation as the respective proteins. To address the molecular mechanisms responsible for this coordinate control, the genes encoding C and R subunits have been isolated and their regulatory regions analysed. Both promoters lack TATA and CCAAT boxes and present multiple transcription start sites. Using gel retardation assays, differences in protein-DNA interactions, associated with the promoters, have been observed in expressing and nonexpressing cells. Footprinting analyses are now in progress to identify the DNA elements involved in gene regulation.

J 307 THE *dsg* GENE OF *Myxococcus xanthus* SHOWS STRONG HOMOLOGY TO *E coli* TRANSLATION INITIATION FACTOR IF3, Lisa Kalman, Yvonne L. Cheng and Dale Kaiser, Dept. of Biochemistry, Stanford University, Stanford, Calif. 94305

Myxococcus xanthus exchange four extracellular factors, A, B, C and D-signals during fruiting body formation. Mutants DK429 and DK439 are synergizable developmental mutants which are defective in the production of the D-signal. These *dsg* mutants also form small, tan colonies during vegetative growth. The *dsg* gene has been cloned and sequenced. The predicted amino acid sequence of the *dsg* gene product shows greater that 50% identity to the translation initiation factor IF3 of *E. coli* and *B. stearothermophilus*. However, the Dsg protein contains a unique C-terminal extension of 66 amino acids which is not present in either the *E. coli* or *B. stearothermophilus* proteins. The *dsg* gene and the *infC* gene, which encodes IF3 in *E. coli*, also share the unique translation initiation codon; AUU. This codon is believed to be involved in autoregulation of IF3 translation.

We have undertaken a series of experiments to determine whether Dsg may function like IF3 in *M. xanthus*. 1. We showed that like IF3, Dsg is able to restore translational regulation of an *infC-lacZ* protein fusion in an *E. coli infC* strain. 2. The Dsg protein fractionates with *M. xanthus* ribosomes. 3. Dsg can cross react with anti-IF3 antisera. 4. Dsg protein is present during both vegetative growth and development and is essential for viability.

In order to determine whether the C-terminal extension of Dsg was important during vegetative growth or development, we have made a series of deletion mutants which lack portions of the C-terminal extension. We have introduced them at either the *dsg* locus or the MX8 att site in a *dsg* strain.

J 308 ANALYSIS OF THE ROLE OF *ORF1590* IN SPORULATION OF *STREPTOMYCES GRISEUS*. Kathleen E. Kendrick, Amitha Dharmatilake, and Lee Ann McCue, Department of Microbiology, Ohio State University, Columbus, OH 43210.

The *orf1590* gene was isolated from *Streptomyces griseus* genomic DNA on the basis of its ability to restore sporulation to Class III bald mutants of *S. griseus*. Nucleotide sequence and transcript analysis revealed the potential to encode two polypeptides, the shorter one of which is N-terminally truncated relative to the longer polypeptide and would therefore lack a putative DNA-binding domain. To determine whether one or two polypeptides are encoded by *orf1590*, we have generated several site-directed mutations in *orf1590* and are assessing the ability of each to restore sporulation to Class III mutants. Initial results indicate that some mutant alleles that alter the potential helix-turn-helix domain of ORF1590 no longer complement the bald mutants. We are currently analyzing mutations that are expected to prevent synthesis of the longer polypeptide as well as other mutations that should prevent synthesis of the shorter polypeptide. The results of these studies will be presented. Previous experiments suggested that the product of *orf1590* may be a DNA binding protein that plays a role in the proper timing of sporulation septation. To identify a potential target of ORF1590, we isolated a 1.4 kb *Sau3AI* fragment that gave rise to a bald phenotype when present in the wild-type strain of *S. griseus* at high copy number. Retransformation confirmed that the bald phenotype was due to the plasmid. Analysis of this transformant revealed that it sporulated poorly on glucose-ammonia minimal medium and no longer produced streptomycin but retained the ability to crossfeed other bald mutants to sporulate, thus showing the characteristics of a Class III phenotype. At high copy number this cloned DNA also prevents sporulation of *Streptomyces lividans*. We will present the results of subclone analysis and nucleotide sequencing of this DNA fragment.

J 309 USE OF GENES UNDER CONTROL OF THE CIRCADIAN CLOCK IN A MUTANT SELECTION SCHEME, Kristin M. Lindgren, Jay C. Dunlap, and Jennifer J. Loros, Department of Biochemistry, Dartmouth Medical School, Hanover NH 03755

It is clear from a cursory examination of the biology of eucaryotes that a wide variety of cellular and metabolic processes are being controlled by biological clocks. We are using *Neurospora crassa* to study the flow of information from the clock to timed target genes- "clock-controlled-genes"- whose levels of expression are controlled on a daily basis by the clock. As a first step, we undertook the systematic isolation of morning and evening specific genes through the use of subtractive hybridization[1]. Two genes, *ccg-1* and *ccg-2*, were identified and found to be abundantly expressed in the subjective morning. Nuclear run-on assays demonstrate that regulation of mRNA abundance for the two morning specific genes occurs at the level of transcription[2]. Sequence analysis of *ccg-1* genomic and cDNA clones reveals that *ccg-1* is identical to a previously described gene, *grg-1* (glucose repressible gene -1). Deletion analysis of upstream *ccg-1* sequences indicates that the glucose and clock regulatory elements are distinct and separate. Localization of the sequences required for clock control of mRNA abundance for the *ccg-1* gene has facilitated the development of a mutant selection/enrichment scheme, in which a selectable drug resistance marker (hygromycin phosphotransferase) has been transcriptionally fused to the *ccg-1* promoter with the goal of driving rhythmic clock regulated drug resistance. Pulses of hygromycin administered at different times during the circadian cycle would allow for the enrichment of new circadian mutant alleles.

[1]Loros, J.J., S.A. Denome, and J.C. Dunlap. 1989 Science 243:385-388.
[2] Loros, J.J., and J.C. Dunlap 1991 Mol. Cell. Bio. 11:558-563.

J 310 STUDIES ON THE ISOLATION, PURIFICATION, CHEMICO-PHYSICAL PROPERTIES AND CHEMICAL STRUCTURE DETERMINATION OF MEILINGMYCIN, L. Ouyang, Y. Gao, and G. Tu, Institute of Microbiology, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, People's Republic of China

Authors reported in 1988 that nanchangmycin A, one of the most effective antibiotic against Coccidia, Toxoplasmea and some insects, was isolated from the fermentation broth of Streptomyces nanchangen -sis n.sp. Yen et Ouyang. Thereafter, another even more effective insecticidal antibiotic was isolated from the fermentation broth of the same micro-organism. More than 30 species belonging to 22 families were tested, the results shew that many of them were sensitive to this antibiotic. Rhabditis and some other nematoes shew also sensitive. The purified antibiotic was colorless slice-shapped crystal. Molecular weight 626, molecular formula $C_{36}H_{51}O_9$. It was soluble in mathanol, chloroform, dichloromethane, acetone, ethyl acetate; less soluble in petroleum ether, normal haxane; insoluble in water. UV-spectrum: λ_{max}^{MeOH} 236, 243, 254(shoulder), IR spectrum: V_{max}^{KBr}(cm) 3460, 2390, 1715, 1650, 1450, 1160-1000. The analysis of UV- and IR-spectrum, ^1H-NMR spectrum and $^{13}C-NMR$ spectrum, $^{13}C-{}^1H$ two dimensional correlation spectrum, $^1H-{}^1H$ two dimensional correlation spectrum and EI-MS indicated that this antibiotic is a 16-membered macrolide antibiotic. Its core structure is identical with that of avermectins but with quite different side chain. There is no glucose residue on C-13 but it possesses a $(CH_3)_2$ CCHCOO-residue on that C atom of 4a position, so it is a new one of 16-membered macrolide antibiotics. It was named meilingmycin. The micro-organism was isolated from Meiling, Nanchang, China.

J 311 A MUSHROOM-INDUCING GENE IN *SCHIZOPHYLLUM*, Carlene A. Raper, J. Stephen Horton, Lisa J. Vaillancourt and Kevin J. Laddison, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT, 05405.

A gene called *Frt1*, isolated from a haploid homokaryon of *Schizophyllum commune*, induces the development of fruiting bodies (mushrooms) when integrated into the genome of other haploid homokaryotic strains which do not otherwise fruit. This gene appears to override the normal requirement of dikaryosis via a compatible mating interaction to induce fruiting. It also has an enhancing effect on dikaryotic fruiting. Combined results from transformation experiments, Mendelian genetics and Southern blot analyses suggest that *Frt1* acts in trans and exists in different allelic states in different strains. The mushroom-inducing activity of endogenous *Frt1* is influenced by activity of the mating-type genes and also appears to involve some form of self/non-self recognition through interalllelic interactions. Spontaneous deletion of the *Frt1* region of the genome in homokaryotic strains in which the mating-type genes are mutated to constitutive function (dikaryon *mimics* that fruit) is correlated with failure to fruit. Such deletion mutants cannot be complemented by transformation with *Frt1*, indicating a requirement for at least one other gene located within the deleted region for fruiting. The *Frt1* gene hybridizes to a 650 nt transcript derived from the strain of origin. Anaysis of the cDNA sequence suggests that *Frt1* encodes a membrane associated protein of 24 kD. Models explaining *Frt1* activity will be discussed.

J 312 THE ROLE OF GUANOSINE PENTA- AND TETRA-PHOSPHATE IN FRUITING BODY DEVELOPMENT IN *MYXOCOCCUS XANTHUS*. Mitchell Singer and Dale Kaiser, Department of Biochemistry, Stanford University, Stanford CA 94305

When *M. xanthus* cells are starved for any of a variety of nutrients and placed at high cell density on a solid support media, cells initiate a developmental program that culminates in the formation of a fruiting body containing environmentally resistant myxospores. Our goal is to identify the primary signals that allows *M. xanthus* to recognize starvation conditions. Our approach is to re-examine the potential role of the known metabolic regulator guanosine penta- and tetra- phosphate (pppGpp and ppGpp). It has previously been shown by Manoil that those conditions that induce sporulation and development in *M. xanthus* and those conditions that induce the synthesis of pppGpp and ppGpp are correlated. We therefore want to directly determine if a rise in the intracellular (p)ppGpp concentration can act as a starvation signal and can initiate the developmental process.

To examine this question we required a method that would allow us to increase the intracellular (p)ppGpp concentrations without employing starvation conditions. The method of choice was to over produce the *E. coli* (p)ppGpp synthetase, RelA protein, from an *M. xanthus* regulated promoter. We have cloned the *E. coli* wild type *relA* gene under the control of the *M. xanthus* light inducible *carR* promoter, previously characterized by D. Hodgson. We report the effect of over-producing (p)ppGpp on *M. xanthus* development.

In addition we have begun characterizing a mutant of *M. xanthus*, DK527, which is unable to accumulate (p)ppGpp in response to starvation. This mutant is blocked very early in the developmental pathway and shows a relaxed rRNA synthesis response to amino acid starvation. Finally, the developmental block can be alleviated by over producing (p)ppGpp in the mutant. This suggests that the inability to accumulate (p)ppGpp may be responsible for the developmental block in the mutant.

J 313 BACILLUS SUBTILIS SIGMA E CHANGED TO SIGMA B SPECIFICITY, Kathleen M. Tatti and Charles P. Moran Jr., Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA. 30322

During sporulation in Bacillus subtilis, new RNA polymerase sigma factors direct the transcription of genes that are required for this cellular differentiation. Genetic and biochemical evidence suggest that the sigma subunit of RNA polymerase determines the specificity of promoter utilization by making sequence-specific contacts with promoter DNA. The amino acid sequences in the region of the sigma factors implicated in genetic suppression studies to contact the -10 region of their cognate promoters are highly conserved in most sigma factors, including those induced during sporulation (e.g., σ^E). This observation suggests that these sigma factors use a similar motif to interact with the -10 regions of promoters. Recently, we showed that an amino acid substitution in the region of σ^E (position 124) that is conserved with the -10 binding region of other sigmas changes the specificity of the interaction of this sigma with the -10 region of its cognate promoters. We now report another change-of-specificity substitution in this region. We substituted Gly and Ile at positions 120 and 119, respectively, to make σ^E more like σ^B, a secondary sigma factor from B. subtilis that is not required for sporulation. We found that this double mutant sigma directs transcription from the σ^B-dependent promoter, ctc. Using a strain in which the gene for σ^B was deleted, we found that a ctc-lacZ fusion produced 90 Miller units of β-gal when the mutant σ^E was induced. The mutant sigma did not use the σ^E-dependent promoters spoIID and spoIIID, and did not support sporulation. These results suggest that the amino acid residues at positions 120 or 119 in σ^E help to determine promoter specificity.

Environmental Sensing and Signal Transduction; Spatial Controls of Gene Expression

J 400 ANALYSIS OF THE INTERACTIONS BETWEEN THE DEVELOPMENTAL REGULATOR *abaA* AND ITS TARGETS IN *Aspergillus nidulans*. Alex Andrianopoulos and William E. Timberlake, Department of Genetics, University of Georgia, Athens, GA, 30602.

Asexual development in the ascomycete *Aspergillus nidulans* requires the action of a number of genetically defined regulatory genes of which the *abaA* gene represents the central component in the defined linear regulatory pathway. Loss of function mutations in the *abaA* gene lead to an arrest of development at the primary sterigmata stage (metula) and reiteration of this cell type.

The predicted amino acid sequence of *abaA* contains a new and novel DNA binding motif designated ATTS (also called TEA), which is also present in the mammalian transcription factors *TEF-1* and *MCBF*, the *D. melanogaster* developmental regulator *scalloped* and the *S. cerevisiae* Ty1 regulator *TEC1*.

DNA-protein interaction studies have identified AbaA binding sites in the *cis*-acting regulatory regions of a number of developmentally regulated genes, including the *brlA*α and *wetA* genes which appear to be upstream and downstream regulators of asexual development, respectively. *In vivo* studies using a heterologous system have shown that these binding sites mediate AbaA-dependent transcription activation. A number of these genes are also targets for the *brlA* regulatory gene products. Potential *in vivo* interactions between these regulators were examined.

These results and the predicted regulatory interactions involved in *A. nidulans* development are discussed.

J 401 REGULATION OF THE GENE ENCODING 6-PHOSPHOGLUCONATE DEHYDROGENASE DURING HETEROCYST DEVELOPMENT IN *ANABAENA* SP. PCC 7120, Stephanie E. Curtis and Patricia J.B. Ligon, Department of Genetics, North Carolina State University, Raleigh, NC 27695

The enzyme 6-phosphogluconate dehydrogenase is the third enzyme in the oxidative pentose pathway, the major route of glycogen breakdown in cyanobacteria and the major source of reductant for nitrogen fixation. The activity of this enzyme is much greater in heterocyst cells than in vegetative cells of *Anabaena*. We are studying the regulation of the gene encoding 6-phosphogluconate dehydrogenase (*gnd*) during heterocyst development in *Anabaena* sp. PCC 7120. The *Anabaena gnd* gene is expressed as three monocistronic mRNAs of approximately 1.6 kilobases. Two transcripts from vegetative cells that differ in length by 48 bases at the 5' end were identified. The 5' termini of each of these transcripts maps just 3' to a "-10" consensus sequence for *Anabaena* promoters. *Anabaena* vegetative filaments were starved for fixed nitrogen and *gnd* transcript levels were analyzed at 6 hr intervals. By 6 hrs after nitrogen starvation, the steady state level of *gnd* transcripts was shown to increase approximately twofold over the level in non-starved cells. Concomitant with this increase was the appearance of a transcript with a unique 5' terminus; this terminus also maps just 3' to a "-10" consensus sequence for *Anabaena* promoters. These results are consistent with an increase in *gnd* transcription derived from a starvation induced promoter. By 36 hrs after nitrogen stepdown, a second increase in *gnd* transcripts to a level approximately fivefold greater than the level in nonstarved cells was observed. No new transcripts were observed at 36 hrs, and thus the increase in transcript level at this time may result from increased transcription from promoters used earlier in development and/or transcript stabilization. At 36 hrs after nitrogen starvation, the *Anabaena* filaments contained heterocysts differentiated at approximately ten cell intervals. The second increase in *gnd* transcript levels late in development is correlated with the maturation of heterocysts and the induction of nitrogen fixation. Although we assayed *gnd* transcript levels in filaments containing both vegetative and developing heterocyst cells, an intriguing possibilty is that both the utilization of a new promoter early in development, and the increase in transcripts late in development are specific to developing heterocyst cells of the filament. These possibilities are currently under investigation.

J 402 ANALYSIS OF COMPLEMENTARY CHROMATIC ADAPTATION IN THE CYANOBACTERIUM *FREMYELLA DIPLOSIPHON* USING *IN VIVO* AND *IN VITRO* FOOTPRINTING AND TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS, Nancy A. Federspiel, Cheryl Schmidt-Goff, and Tracy Ridgeway, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843.

Cyanobacteria such as *Fremyella diplosiphon* alter the protein composition of their light-harvesting organelles, the phycobilisomes, in response to light quality, a process known as complementary chromatic adaptation. In addition, morphological and developmental changes can be stimulated by these changes in the wavelength of light. We have analyzed the promoter of the green light-activated phycoerythrin operon *cpeBA* using *in vivo* dimethyl sulfate footprinting and have identified two guanosine residues which are protected in *F. diplosiphon* cultures grown in both red and green light. These residues are also protected in four different pigmentation mutants. We have partially purified this putative transcription factor, designated RlrA (for Regulator of the Light Response) from extracts of cultures grown under both light regimes; *in vitro* footprinting with dimethyl sulfate and with DNase I defined a region from -45 to -65 bp upstream of the transcription start site which is protected by RlrA binding. These results imply that RlrA is continuously bound to the *cpeBA* promoter in both red and green light; differential gene expression could be achieved by RlrA modification and/or interaction with another factor. We are analyzing other cellular changes which are influenced by light quality using two-dimensional polyacrylamide gel electrophoresis of protein extracts of *F. diplosiphon* cultures grown in red or green light. While the majority of proteins visible by silver staining are common to both light regimes, quantitative and qualitative differences can be seen in the protein profiles of soluble, phycobilisome, and membrane fractions. Pulse labeling of cultures switched from one light regime to the other identify unique rapidly labeled proteins which may be important in the differential regulation in response to light quality or in morphological and developmental changes.

J 403 CLONING AND MOLECULAR CHARACTERIZATION OF *apsA* (= ANUCLEATE PRIMARY STERIGMATA), A GENE NECESSARY FOR DEVELOPMENT IN *Aspergillus nidulans*, Reinhard Fischer and William E. Timberlake, Department of Genetics, University of Georgia, Athens, GA 30602

Aspergillus nidulans is an ascomycete which is able to reproduce sexually and asexually. For asexual development this fungus forms conidiophores, consisting of a stalk, a vesicle, primary and secondary sterigmata and several hundred conidia, each containing one nucleus (Timberlake 1990). In *apsA* mutants the nuclei fail to migrate into the primary sterigmata. When a nucleus fortuitously enters the sterigmatum the development proceeds and a single chain of conidia is formed (Clutterbuck 1969), showing that the *aps* function is restricted to a single developmental stage.

The *apsA* mutation was complemented by transforming *A. nidulans* strain RF1 (*apsA1; trpC801; pabaA1; wA3; yA2*) with a cosmid library with *trpC* as the selective marker. A single cosmid was recovered from a transformant which then complemented the mutation at high frequency. The *apsA*-gene was localized in a 10.8 kb *BamHI* fragment within the 40 kb insert by cotransformation with subcloned restriction fragments. This region encoded 1.6 kb and 6.2 kb transcripts. Neither transcript was developmentally regulated. Gene disruption and RNA blot analyses showed that the larger transcription unit corresponds to *apsA*.

Literature

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Timberlake W.E. (1990) Molecular genetics of *Aspergillus* development. Ann.Rev.Genet. 24:5-36.

J 404 IDENTIFICATION, CLONING, AND SEQUENCING OF A NOVEL EARLY SPORULATION GENE IN *Bacillus thuringiensis*, Thomas Malvar, Cynthia Gawron-Burke, and James A. Baum, Ecogen Inc., 2005 Cabot Blvd. West, Langhorne, PA 19047.

The *Bacillus thuringiensis* (B.t.) strain EG1351 harbors a spontaneous mutation which causes a defect in sporulation (sporulation frequency ~$1x10^{-7}$). Primer extension analysis of total RNA from EG1351 failed to detect *sigG* or *sigK* (*sig28*) specific mRNA whereas both RNAs were detected in the parental wild type strain EG2158. Similar analyses showed that *spo0A* transcription is regulated the same in EG1351 and EG2158. This result suggests that the defect in EG1351 negatively effects the activation of *spo0A* and may occur early in sporulation.

The defective gene (*fun1*: function unknown) in EG1351 was cloned by complementation of function and localized to a 1224 bp open reading frame. This predicts a protein with an estimated molecular weight of 47.7 Kdal. The *fun1* protein shows the most similarity to the kinase *spoIIJ* (*kinA*) from *B. subtilis* (25% identity) as well as to other sensor proteins belonging to a two component signal transduction system. Notably, the C-terminal portion of *fun1* shows the highest degree of similarity to the corresponding region of *spoIIJ*, particularly in two regions (A/S-H-E-I-K/R-T/N-P-L and G-T-G-L-G-L) which are highly conserved in the sensor proteins.

These similarities suggest that *fun1* may play a role similar to *spoIIJ*. The observation that defects in *fun1*, unlike *spoIIJ*, dramatically affect the sporulation frequency implies a more centralized role for *fun1* in the onset of sporulation.

J 405 GENETIC AND MOLECULAR CHARACTERIZATION OF THE *Aspergillus nidulans wA* GENE, María E. Mayorga and William E. Timberlake, Department of Genetics, University of Georgia, Athens, GA 30602

The purpose of this work is to contribute to the understanding of the regulatory mechanisms controlling gene expression during asexual development (conidiation) in the ascomycete *Aspergillus nidulans*. This work focuses on characterization of the developmentally regulated *wA* gene. The *wA* gene has been cloned, its pattern of transcript accumulation characterized and its physical structure elucidated (Mayorga and Timberlake, Genetics, **126**, 73-79, 1990). The *wA* gene codes for a putative polyketide or fatty acid synthase and appears to be involved in the synthesis of the green pigment present on the walls of mature conidia (Mayorga and Timberlake, Mol. Gen. Genet, in press).

Characterization of the *cis*-acting regulatory regions of *wA* should provide information about late gene regulation and contribute to the understanding of the regulatory network that controls conidiophore development and conidium differentiation. Of three well characterized *A. nidulans* developmental regulators, two, *abaA* and *wetA*, are implicated in the regulation of *wA*. Mischeduled expression of *wetA* results in the accumulation of *wA* message (Marshall and Timberlake, Mol. Cell. Biol., **11**, 55-62, 1991). In addition, 800 bp of *wA* upstream sequences that are sufficient to confer develpmental regulation on a reporter gene contain binding sites for the product of *abaA*. The results of the interactions of these two putative regulatory genes with *wA* upstream sequences are described in this poster.

J 406 THE *med*A GENE OF *ASPERGILLUS NIDULANS*,
Karen Y. Miller, Jianguo Wu, and Bruce L. Miller, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Id. 83843

The *med*A and *stu*A genes of *Aspergillus nidulans* are responsible for the correct spatial patterning of the asexual reproductive structure, the conidiophore. They along with three additional genes, *brl*A, *aba*A, and *wet*A are necessary for normal conidia production. *med*A and *stu*A gene products are also essential for sexual reproduction, with null mutants being infertile. Northern blot analysis of *med*A mutants show that the abundance and temporal expression of the *stu*A transcript is unaffected. However, *brlA* transcripts are prematurely expressed and *aba*A RNA abundance is reduced. We have cloned *med*A by complementation and sequenced both the genomic and cDNA sequences. The *medA* RNA is similar to the *stu*A RNA structure in that it has a long untranslated leader with a number of small open reading frames (ORFS). The transcriptional and translational regulation of *med*A will be discussed.

J 407 Expression of the *spi*A gene reveals a spatial gradient during *Dictyostelium* sporulation.
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*spi*A is a prespore specific gene which is expressed only during culmination of *Dictyostelium discoidium*. Its message accumulates in prespore cells and spores during sporulation, and subsequently disappears as the spore matures. The gene was disrupted by gene replacement, and the mutant strains develop normally to produce spores which are initially indistinguishable from wild-type. However, *spi*A$^-$ spores are unstable when exposed to water in the environment and lose viability much more rapidly than wild-type. The *spi*A gene product, Dd31, was identified on the inner face of the spore coat by using antibodies prepared against recombinant Dd31 fusion protein.

Unlike other spore coat genes, which are expressed in prespore cells for approximately 10 hours prior to sporulation, *spi*A is expressed briefly during sporulation. We isolated the promoter region of *spi*A, fused it to the *E. coli lacZ* gene, then stably transformed the promoter fusion construct into *Dictyostelium*. During development, β-galactosidase expression coincides with the expression of Dd31 protein, which begins as the ball of prespore cells reaches about two thirds of the way up the newly formed stalk. Blue staining first appears at the top of the prespore mass, with a gradient of decreasing blue extending a short way downwards into the prespore mass. As culmination proceeds, the gradient deepens and extends further down the prespore mass, until at completion of culmination the entire spore mass stains evenly blue. A series of *spi*A promoter deletion constructs fused to *lacZ* are presently being analyzed to identify the cis-acting elements necessary for the spatial and temporal regulation during sporulation.

J 408 HETEROGENEITY OF THE PRINCIPAL SIGMA FACTOR OF *Escherichia coli*: THE *rpoS* GENE PRODUCT, σ^{38}, IS A PRINCIPAL SIGMA FACTOR OF RNA POLYMERASE IN STATIONARY PHASE *Escherichia coli*
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RNA polymerase holoenzyme containing the principal sigma factor σ^{70} (the *rpoD* gene product) is responsible for the transcription of almost all genes expressed in exponentially growing cells of *Escherichia coli*. Reconstituted RNA polymerase holoenzyme containing σ^{38} (the *rpoS* gene product) was found to recognize *in vitro* many typical σ^{70}-type promoters including *lac*UV5, *trp* and RNA I promoters. Some promoters, however, were recognized exclusively or preferentially by $E\sigma^{70}$ while at least one promoter was favored by $E\sigma^{38}$. Thus *E.coli* promoters can be classified into three groups: the first in recognized by both $E\sigma^{70}$ and $E\sigma^{38}$; the second is preferred by $E\sigma^{70}$; and the third is recognized by both $E\sigma^{70}$ and $E\sigma^{38}$ alone. In contrast to other minor sigma factors, σ^{38} shares basic amino acid sequences common among the principal sigma factors of eubacteria, and is therefore a member of the *rpoD*-related gene family. These results together indicate that σ^{38} is a second principal sigma factor, present in stationary phase *E.coli*.

Late Abstract

CHARACTERIZATION OF ENTRY INTO STATIONARY PHASE IN THE YEAST *SACCHAROMYCES CEREVISIAE*.
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Stationary phase is a relatively poorly understood phase of the yeast growth cycle characterized by distinct morphological, physiological, and biochemical alterations. Our laboratory is interested in studying entry into stationary phase as a developmental process in yeast. The goal of our research is to identify genes that regulate entry into stationary phase or that are expressed in stationary phase and to characterize stationary phase-specific biochemical changes. We have determined, after careful growth studies, that yeast cells enter stationary phase only after one week in rich, glucose-based liquid medium at either 24 or 30°C. This is in direct contrast with the commonly-held belief that yeast cells enter stationary phase after one or two days in liquid cultures. We have observed dramatic increases in both the modification and abundance of Bcy1p, the regulatory subunit of cAMP-dependent protein kinase, after two days and again after one week of culture. We have used a gene, the *HSP70*-related, *SSA3* gene, which is expressed only after two days in liquid culture, to select for mutations that affect gene expression after exponential growth slows but prior to entry into stationary phase. The *AAP1* gene, which complements a defect in *SSA3* expression, has been cloned and sequenced and encodes a novel alanine/arginine aminopeptidase whose activity is related directly or indirectly to glycogen accumulation. We are currently characterizing the stationary phase-specific modifications of Bcy1p, cloning additional genes involved in *SSA3* expression, and identifying genes whose mRNAs accumulate in stationary phase yeast cells. The results of these studies will be presented.