MOLECULAR GENETICS OF FILAMENTOUS FUNGI

William Timberlake, Organizer

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Transformation and Gene Manipulation

1554 MITOCHONDRIAL PLASMIDS AND TRANSFORMATION OF NEUROSPORA. Alan M. Lambowitz, Dept. of Biochemistry, St. Louis University Medical School, St. Louis, MO 63104

Mitochondrial plasmids are small circular or linear DNAs that are found in mitochondria, but are not homologous to the standard mtDNA. Neurospora mitochondrial plasmids are not present in standard laboratory wild-type strains, but are relatively common in natural isolates. In screening experiments, we identified four different plasmids in four Neurospora strains: Mauriceville, Fiji, Labelle, and Varkud. These plasmids are all closed circular DNAs and range in size from 3.6 to 5.2 kb. The Mauriceville, Fiji, and Varkud plasmids belong to three different classes, whereas the Varkud plasmid belongs to the same class as the Mauriceville plasmid (1,2).

To gain insight into the origin and biological role of mitochondrial plasmids, we have completely sequenced the Mauriceville mitochondrial plasmid (3.6 kb) and have mapped its major transcripts (3). The plasmid contains a long open reading frame that is expressed in its major transcript and that could encode a hydrophilic protein of 710 amino acids. Two characteristics of the plasmid--codon usage in the long open reading frame and the presence of conserved sequence elements--suggest that it is related to Group I mitochondrial introns. The major transcripts of the plasmid are full length linear RNAs that have heterogeneous 5' ends and a single major 3' end. The major 5' and 3' ends are adjacent and slightly overlapping. The sequence and pattern of transcription of the plasmid suggest that it may belong to a class of genetic elements that were or are the progenitors of mtDNA introns.

The Varkud plasmid (3.8 kb) is closely related to the Mauriceville plasmid, judged by DNA-DNA hybridization and restriction enzyme mapping. The predominant transcript of the Varkud plasmid has a length of 5.3 kb, approx. 1.5 kb longer than the monomer length of the plasmid. Mapping of Varkud transcripts by R-loop, S1 nuclease, and primer extension methods suggests that the 5.3 kb RNA is a "wrap-around" formed by reading through a stop site during the first transcription passage. This pattern of transcription leads to an RNA with a terminal direct repeat. The presence of such a transcript provides additional suggestive evidence that the plasmids are related to mobile elements.

To investigate directly whether mitochondrial plasmids can function as infectious or mobile elements, the Mauriceville, Varkud, and Labelle mitochondrial plasmids have been introduced into Neurospora host strains as constituents of recombinant plasmids or by cotransformation with recombinant plasmids containing genetic markers selectable in Neurospora.

(1) Collins, R.A., Stohl, L.L., Cole, M.D. and Lambowitz, A.M. Cell 24, 443-452, 1981.
(2) Stohl, L.L., Collins, R.A., Cole, M.D. and Lambowitz, A.M. Nucl. Acids. Res. 10, 1439-1458, 1982.
(3) Nargang, F.E., Bell, J.B., Stohl, L.L. and Lambowitz, A.M. Cell 38, 441-453, 1984.

1555 VECTORS FOR THE ISOLATION OF GENES BY EXPRESSION IN ASPERGILLUS NIDULANS, Geoffrey Turner, Department of Microbiology, University of Bristol, Bristol BS8 1TD, U.K.

Shotgun cloning into Aspergillus in order to isolate genes by expression requires a relatively high frequency of transformation combined with an adequate insert size in the vector. At the time of writing, there is no convincing evidence for autonomous replication in Aspergillus of any of the transforming vectors in current use, but chromosomal integration occurs readily at different sites, even in the absence of good homology between transforming DNA and chromosomal DNA. The integrated plasmid and flanking chromosomal sequences can easily be rescued by transformation of E. coli with DNA isolated from the Aspergillus transformant.

While any gene which can be selected for in Aspergillus can in theory transform, the frequency obtained is generally too low for gene isolation by shotgun cloning. A number of groups have increased the frequency of transformation by reconstruction of simple vectors based on a gene which can be selected in Aspergillus cloned into a bacterial plasmid or cosmid, but the reasons for the frequency increases are not immediately apparent.

We have incorporated into our simple vectors a 3.3 kb Aspergillus chromosomal fragment (ans-1) which results in approximately 50 fold increase in transfomation frequency. Although this fragment was isolated by its ability to confer replication on an integrative plasmid in yeast, we have no evidence that it does the same in Aspergillus. Probing of the genomic DNA with this fragment identifies the native sequence and, in addition, several other sequences with some lesser homology. Recent studies on these sequences will be reported.

We have also investigated an Aspergillus mitochondrial DNA fragment which increases transformation frequency about 10-fold.

While most directly selectable markers now available in Aspergillus are nutritional, and require the appropriate mutant as a recipient strain, we have recently been using an antibiotic resistance selection system based on an oligomycin-resistance gene coding for the proteolipid of the mitochondrial ATP synthetase. This gene may prove useful in gene replacement methodology. When present in low copy number together with the wild-type gene, the organism shows a low level of resistance, but some of these strains spontaneously give rise to high level resistant derivatives at high frequency. The molecular basis of this phenomenon will be discussed.

1556 THE CURRENT STATUS OF TRANSFORMATION IN THE BASIDIOMYCETE SCHIZOPHYLLUM COMMUNE, Robert C. Ullrich*, Charles P. Novotny*, Charles A. Specht*, Eunice A. Froeliger+, and Alfredo R. Muñoz-Rivas*. Departments of Botany* and Medical Microbiology+, University of Vermont, Burlington, VT 05405

Schizophyllum commune is a woodrotting Basidiomycete with a well-defined mating system controlled by two sets of mating-type genes, A and B. Our interest is to develop the transformation system needed to isolate these mating-type genes and other genes of interest. The Basidiomycetes represent a large and economically important class of fungi; transformation in Schizophyllum would be a useful tool for molecular genetic studies, and the methodology will be useful to those working on other Basidiomycetes. We have been attempting to transform Schizophyllum protoplasts using naked DNA or DNA encapsulated in synthetic liposomes. These experiments used vectors carrying identified genes from other organisms plus sequences of Schizophyllum DNA. In each case the selection and identification of putative transformants depended upon the activity of these foreign genes. Schizophyllum sequences included in many of the vectors were either 1) random sequences shotgunned into the vectors in order to aid replication of the vector or promote transcription from the foreign gene, or 2) sequences selected on the basis of their providing these functions in Saccharomyces cerevisiae. Other Schizophyllum fragments were shotgunned into specific constructions of plasmid vectors in order to increase the likelihood of integration of transforming DNA into the genome. We have also used the neomycin-resistance gene (neo^r) of transposon Tn5 in various vectors. Neo^r codes for an aminoglycoside phosphotransferase that confers resistance to several aminoglycosides including the toxic antibiotic G418 (i.e., Geneticin). Others have used neo^r to transform mammalian cells, plant cells and the slime mold Dictyostelium to G418^R. Although some Schizophyllum transformants appear, the transformation occurs at low frequency, appears to be unstable, and is confounded by spontaneous mutations to G418^R. Because we have been unable to identify a suitable selective marker from Schizophyllum by complementation of auxotrophic mutations in S. cerevisiae or E. coli, we have invested in two alternative approaches: one using immunology and another using cross-hybridizations. The talk will update and amplify these results and offer suggestions for developing transformation in Basidiomycetes based on our experience with Schizophyllum.

1557 DEVELOPMENT OF A SYSTEM FOR ANALYSIS OF REGULATION SIGNALS IN ASPERGILLUS, [1]C.A.M.J.J. van den Hondel, [1]R.F.M. van Gorcom, [2]T. Goosen, [2]H.W.J. van den Broek, [3]W.E. Timberlake, [1]P.H. Pouwels, [1]Medical Biological Laboratory TNO, Rijswijk, The Netherlands, [2] Dept. of Genetics, Agricultural University, Wageningen, The Netherlands, [3]Dept. of Plant Pathology, University of California, Davis, USA.

Regulation of gene expression in Aspergillus nidulans has been investigated extensively by genetic methods. However, little is known about control of gene expression at the molecular level. A very convenient and powerful system to study gene expression is provided by the fusion of a promoter or regulatory region to the lacZ gene of E. coli, encoding β-galactosidase, and determination of the β-galactosidase activity.

To develop such a system for A. nidulans we have constructed in vitro a fusion of the E. coli lacZ gene and the trpC gene of A. nidulans. The fused gene was cloned into a plasmid which contains an A. nidulans selection marker. Analysis of A. nidulans strains transformed with this plasmid shows that the E. coli lacZ gene is expressed as a functional fusion protein.

The system will be used to analyse transcription-regulation signals of inducable Aspergillus genes or genes which are expressed constitutively in A. nidulans.

The results obtained in A. nidulans with the trpC-lacZ fusion gene system and its potential applications will be discussed.

Transformation and Gene Manipulation

1558 MOLECULAR CLONING OF THE A.NIDULANS ISOCITRATE LYASE GENE BY EXPRESSION IN A.NIDULANS, D.J.Ballance and G.Turner, University of Bristol, Bristol, U.K.

An Aspergillus nidulans gene library was constructed in the high transformation efficiency vector pDJB3, which is based on the Neurospora crassa PYR4 gene and carries an A.nidulans sequence, ansl. DNA from this library was used to transform a pyrG ; acuD double mutant of A.nidulans, selecting both for uridine-independence and for acetate-utilization. DNA prepared form two of the four transformants was subjected to partial digestion with EcoRI followed by ligation and then transformation of E.coli. A proportion of the rescued plasmids were able to retransform the acuD strain to acetate-utilization. These plasmids were also able to transform all other acuD alleles tested, including three temperature-sensitive mutants. Analysis of the isocitrate lyase gene is in progress; up to date results will be presented.

1559 ANALYSIS OF MITOCHONDRIAL GENOME AND PLASMID-LIKE DNA FROM FUSARIUM SPECIES, Daniel Cullen, Allen Budde, Harold Kistler, Deborah Samac, and Sally Leong, University of Wisconsin, Madison 53706

Mitochondrial DNA was purified from 4 Fusarium sporotrichioides and 3 F. tricinctum strains. Restriction endonuclease digests were compared by agarose gel electrophoresis. No clear relationship was observed between taxonomic position and restriction patterns. Similiarly, the ability of strains to produce trichothecene toxins appeared unrelated to restriction polymorphisms.

An extachromosonal DNA element, approximately 2.1 Kb in size, was repeatedly isolated from the mDNA band of CsCl gradients of a single F. sporotrichioides strain. Southern hybridizations using the element as probe showed no homology with the nuclear or mitochondrial genomes or with a 1.9 Kb plasmid-like DNA from F. oxysporum f. sp. conglutinans. The intramitochondrial location of the F. sporotrichioides element was demonstrated by examining DNA purified from isolated (DNAse treated) mitochondria.

1560 CLONING AND CHARACTERIZATION OF THE MOLYBDENUM COFACTOR GENES nit-9A AND nit-9B OF Neurospora crassa; Nigel Stuart Dunn-Coleman; Present address: Visiting Scientist, Central Research and Development Department, Du Pont Experimental Station, Wilmington, Delaware, 19898, USA

Molybdenum cofactor (MoCo) mutants of Neurospora crassa lack both NADPH:nitrate reductase activity and xanthine dehydrogenase activity. The tightly linked nit-9A, nit-9B and nit-9C, MoCo mutants, when grown with nitrate as the sole nitrogen source and high levels of molybdate, have nearly normal levels of nitrate reductase activity. An N. crassa MoCo gene was previously isolated using an N. crassa genomic library to transform the molybdate repairable Escherichia coli MoCo mutant chlD (Dunn-Coleman 1984. Curr. Genetics 8). Further studies to identify to which N. crassa MoCo mutant the E. coli mutant chlD is functionally equivalent are now reported. The originally isolated pMoCo plasmid (1:4) has now been used to transform at very low frequency the N. crassa MoCo mutants nit-9A and nit-9B. It was not possible to transform the nit-9C mutant with the pMoCo plasmid. When the 1.2 Kb HindIII fragment was deleted from the pMoCo plasmid, only nit-9B transformants were obtained. Both nit-9A and nit-9B transformants have normal levels of nitrate reductase activity. Colony hybridizations and Southern analysis using pBR322 as a probe detected pBR322 sequences in all nit-9A and nit-9B transformants. These results indicate that the pMoCo plasmid encodes the nit-9A and nit-9B MoCo genes.

1561 ANALYSIS OF CIS-ACTING TRANSCRIPTIONAL MUTANTS OF THE INDUCIBLE QA-2 GENE IN NEUROSPORA CRASSA, Robert F. Geever, Mary E. Case, Brett M. Tyler, and Norman H. Giles, University of Georgia, Athens, GA 30602

Expression of the qa-2 gene of N. crassa normally requires a functional activator protein encoded by qa-1F. Twelve transcriptional mutants of the qa-2 gene have been isolated that allow partial expression of qa-2 (1-45% of induced wild-type) in the absence of functional activator protein ($qa\text{-}2^{al}$ mutants). Each mutation has been identified, cloned, and sequenced, and all mutations occur 5' to the qa-2 gene within a 300 bp upstream region. The qa-2 mutations include point mutations (two), small duplications (two), and large DNA rearrangements (8), most of which involve reciprocal exchanges (viz., inversions or translocations). Transcriptional analysis reveals that all mutants preferentially initiate transcription 45 bp upstream from the major inducible site in wild-type, where normally only minor transcription initiation is observed. Restoration of functional $qa\text{-}1F^{+}$ activator protein in one highly active $qa\text{-}2^{al}$ strain, having a rearrangement mutation at -378 bp, results in both constitutive and inducible expression of qa-2. Furthermore, upon induction, transcription proceeds equally from both minor and major qa-2 initiation sites. Work is in progress toward identifying the region of qa-1F activator protein recognition by examining other rearrangement mutants ($qa\text{-}2^{al}$, $qa\text{-}1F^{+}$) in which the location of the breakage site occurs between -80 and -259 bp.

1562 PLASMID-LIKE DNAs IN MUSHROOMS, Paul A. Horgen, Madan Mohan, Robert J. Meyer and James B. Anderson, University of Toronto, Mississauga, Ontario, Canada, L5L 1C6

Two unique plasmid-like DNA components were localized in isolated mitochondria of the commercially important mushroom genus, Agaricus pEM (7.35±0.15kb) and pMPJ (3.65±0.15kb). The DNA moieties were linear; pEM possessed regions of terminal inverted repeated sequences. No homology was detected in hybridizations between pEM or pMPJ and the nuclear or mitochondrial genomes. No homology existed between pEM and pMPJ. This suggests independent replication of pEM and pMPJ. Restriction endonuclease digests indicated that pEM consisted of two components (pEM_1 and pEM_2) with uniquely different restriction sites and copy number. A survey of several wild isolates of A. bitorquis indicates that plasmid-like DNAs are quite common in nature. The potential of the indigenous plasmids for transformation vectors for the commercial mushroom is being investigated.

1563 CLONING GENES FROM CANDIDA ALBICANS, Myra B. Kurtz and Donald R. Kirsch, The Squibb Institute for Medical Research, Princeton, New Jersey 08540

Candida albicans is a dimorphic yeast and an opportunistic pathogen in man. It is believed to be a diploid organism and has no sexual cycle so that genetic analysis is limited to the isolation of mutants following fairly heavy mutagenesis and the analysis of these mutations by laborious parasexual techniques. Cloning Candida genes and studying their structure and expression in heterologous hosts is one way to circumvent the difficult genetics of the organism. The feasibility of this approach has already been demonstrated by the isolation of the Candida albicans gene for orotidine-5'-phosphate decarboxylase by complementation of a ura3 mutation in Saccharomyces cerevisiae (A. Gillum, E. Tsay, and D. R. Kirsch, Mol. Gen. Genetics, in press). We will discuss a similar approach to the isolation of other physiologically important genes from Candida albicans.

1564 LINEAR PLASMID-LIKE DNA IN FUSARIUM OXYSPORUM, H. C. Kistler and S. Leong, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

Small (1.9 kb) linear DNAs have been found in certain isolates of the fungus Fusarium oxysporum f. sp. conglutinans. The DNA element studied from one strain was insensitive to pancreatic RNase A and S-1 nuclease, but sensitive to pancreatic DNase I, exonuclease III and the restriction enzymes AluI and Sau3A. This along with electron microscopic examination indicates that the molecules are linear double-stranded DNA. However, the DNA was insensitive to an exonuclease with 5' → 3' activity, λ-exonuclease, suggesting that the 5' terminus of this molecule is blocked. Southern hybridization analysis indicated no homology between the plasmid and mitochondrial DNA of the strain from which the plasmid was derived. These plasmid-like DNAs have been found in 2 of 5 strains of F. oxysporum and are not associated with slow growth or "killer" phenotypes.

1565 IDENTIFICATION OF A STRAIN OF Neurospora intermedia WITH ATYPICAL AGING PATTERNS, C.J. Myers, A.J.F. Griffiths, U. of British Columbia, H. Bertrand, U. of Regina, Canada.

In most Kauaian isolates, mitochondrially-based senescence is characterized by a consistent onset of aging for individual strains, abnormal cytochrome spectra (excess c and little b or aa_3), and alterations in the mitochondrial DNA. The latter aging characteristic is of special interest because it involves the insertion of a 10kb DNA species (kal DNA) into the intron of the large rRNA gene.

One Kauaian strain under investigation does not show these same aging patterns. This strain was first identified when various cultures, derived from the same stock, exhibited different life-spans. These life-spans ranged from an almost immediate onset of aging to immortality. In aging cultures, abnormal cytochrome spectra and the presence of kal DNA were not consistently observed. When kal DNA was detected, it appeared as an autonomous molecule carrying a small deletion. From Southern hybridizations, it was evident that kal DNA had originally inserted into the intron of the large rRNA gene and later excised generating the autonomous molecule. Particularly interesting, is the presence of kal DNA in the immortal cultures. Only a small part of kal DNA is detected which is inserted into the intron of the large rRNA gene. Deletion of a substantial portion of kal DNA might account for why these cultures have not shown any signs of aging.

Thus, in this strain, it appears as though there is a tendency to modify the inserted kal DNA and that the fate of the culture is dependent on the extent of the deletion to kal DNA.

1566 MITOCHONDRIAL PLASMIDS FROM COCHLIOBOLUS HETEROSTROPHUS. Sally VanWert, Robert Garber, Jim Oard, J.J. Lin, Gillian Turgeon, and Olen Yoder, Cornell University, Ithaca, NY 14853-0331.

I am a postdoctoral fellow in Olen Yoder's laboratory in the Dept. of Plant Pathology at Cornell University. Our research interests are 1) to develop a transformation system for Cochliobolus heterostrophus, a fungal pathogen of corn, 2) to screen and characterize isolates of Cochliobolus for the presence of plasmids which may be useful in vector construction, 3) to characterize the mitochondrial genome of Cochliobolus and compare the results with analysis of the mitochondrial genome of two other filamentous fungi - Neurospora and Podospora. Our long term goal is to clone and study genes involved in pathogenicity.

1567 THE BENOMYL^r GENE OF NEUROSPORA CRASSA: CLONING, SEQUENCE AND TRANSFORMATION, Marc Orbach, Steven Vollmer and Charles Yanofsky, Stanford University, Stanford, CA 94305.

The β-tubulin gene of N. crassa strain Bml^r(511)a was cloned using the A. nidulans gene as a probe. The β-tubulin gene was physically mapped to linkage group VIL, close to the location of the Bml^r locus. The β-tubulin allele from Bml^r(511)a was shown to confer resistance to benomyl in transformation experiments.

Sequence analysis of the β-tubulin gene reveals high homology to the yeast gene at the amino acid level while diverging considerably at the DNA sequence level. The Neurospora gene contains four intervening sequences which have 5' and 3' splice junctions homologous to the junctions of other Neurospora introns.

Bml^r transformants may be selected using as little as 250 ng/ml of the fungicide. The levels of resistance and morphologies of transformants vary considerably whereas strain Bml^r(511)a is virtually unaffected in its growth on media containing from 0.25 - 2.5 µg/ml benomyl. Molecular and genetic analyses of transformants will be presented, as well as results of heterologous transformations. The isolation of a semi-dominant selectable marker for transformation should aid gene-replacement studies and the analyses of genes for which direct selection in transformation is not possible.

1568 DEVELOPMENT OF SHUTTLE VECTORS AND GENE MANIPULATION TECHNIQUES FOR NEUROSPORA CRASSA, John V. Paietta and George A. Marzluf, Dept. of Biochemistry, Ohio State University, Columbus, Ohio 43210

A potential shuttle vector (pJP102) has been isolated from a clone bank containing a selectable marker and Neurospora BamHI chromosomal fragments. The clone bank was used to transform Neurospora and the plasmid DNA was extracted from the resulting transformants which were grown in liquid culture. pJP102 was isolated following transformation of E. coli to ampicillin resistance with the plasmid DNA. From these types of experiments we have also found deletions in plasmids passaged through Neurospora. These deletion derivatives have increased transformation frequencies and may be improved integrating vectors. In addition, we have carried out gene disruption in Neurospora using linear DNA fragments carrying a gene of interest whose coding sequences were interrupted by a selectable marker. In such experiments, linear DNA fragments enhanced the transformation frequency and proportion of gene disruptions. Targeted replacement of a specific region in Neurospora is possible, but complicated due to multiple and apparently non-homologous integrations. The details and uses of the procedure will be described.

1569 EVIDENCE FOR THE EXPRESSION OF THE E. COLI ChlM NITRATE GENE IN N. CRASSA, Virginia B. Patel, Nancy B. Roberts and Mary E. Case, University of Georgia, Athens, GA 30602

A nit-9$^-$ qa-2$^-$ strain of Neurospora crassa has been transformed by plasmid pSC82 containing the qa-2$^+$ gene from N. crassa and the ChlM gene (one of the molybdenum cofactor genes) from E. coli. Evidence is presented for the expression of the ChlM gene in N. crassa. A 750 bp TaqI fragment containing the E. coli ChlM gene has been inserted into the ClaI site of pBR322, plasmid pFG7 (obtained from A. Kleinhofs, Washington State University). Plasmid pSC82 contains the 3.2 kb HindIII fragment from pRC57 which carries the qa-2$^+$ gene from N. crassa inserted into the HindIII site in pFG7. N. crassa recipient strains for transformation were made by crossing the qa-2$^-$ arom-9$^-$ strain to strains carrying the various N. crassa molybdenum cofactor mutations, nit-7, nit-8, and nit-9. Transformants were selected first for expression of the qa-2$^+$ gene and then these transformants were tested for the ability to grow on a low nitrogen source indicating the expression of the ChlM gene. DNA-DNA hybridization analyses of nonhomokaryotic and homokaryotic transformants indicated that both pBR322 and the isolated 750 bp fragment containing the E. coli ChlM gene are present and integrated into the N. crassa genome. DNA-poly(A)$^+$RNA filter analysis of three of the nonhomokaryotic and one homokaryotic isolates indicated the presence of mRNA species hybridizing to the cloned 750 bp E. coli ChlM gene. Although the detected messages which vary in size are larger than the cloned ChlM gene, these results indicate that the cloned ChlM gene from E. coli is transcribed and translated in N. crassa.

1570 TRANSFORMATION OF CEPHALOSPORIUM ACREMONIUM WITH AN ANTIBIOTIC-RESISTANCE MARKER
M.A. Peñalva, A. Touriño, F. Sánchez, C. Patiño, V. Rubio & J.M. Fernández-Sousa
ANTIBIOTICOS, S.A. - MADRID - SPAIN

C. acremonium is a filamentous fungus used for the industrial production of cephalosporin C. This fungus is sensitive to relatively low amounts of hygromycin B and G418. G418 is inactivated by phosphotransferases known to inactivate neomycin and kanamycin by phosphorylation of the antibiotic. We have used a gene coding for an aminoglycoside-3'phosphotransferase encoded by E.coli transposon Tn903 as a dominant marker to select transformants in a medium containing G418. This gene confers neomycin or kanamycin resistance in E.coli as well as G418 resistance in the yeast S.cerevisae. We have been able to transform C.acremonium using a PEG-Ca++ treatment of sphaeroplasts to induce the uptake of a foreign plasmid DNA that contained, in addition to the complete Tn903 gene coding for neomycin (G418) resistance the E.coli plasmid replicon pCD5 and a 1.94Kb C.acremonium mitochondrial DNA fragment that confers ars phenotype in yeast. Sphaeroplasts were plated out onto regeneration medium and, after a 24 hours incubation, selection plates were overlaid with regeneration agar containing enough G418 to give a final concentration of 50ug/ml. 0.5-2 transformants /ug DNA were obtained suggesting that integrative transformation instead of autonomous replication of the plasmid was taking place. Southern analysis of DNA from transformants confirmed this prediction and suggested that integration occurred in a random fashion. The nucleotide sequence of the DNA that confers ars phenotype in yeast has been elucidated and the relevant features of this sequence will be discussed. Experiments designed to increase the level of expression of the inactivating enzyme as well as the frequency of integration of the foreign DNA are in progress.

1571 CLONING OF THE ORNITHINE CARBAMOYLTRANSFERASE GENE FROM NEUROSPORA CRASSA,
Diane Puetz and Richard L. Weiss, UCLA, Los Angeles, CA 90024

The lambda gt11 cloning system is being used to clone the N. crassa gene for ornithine carbamoyltransferase, an arginine biosynthetic enzyme. Ornithine carbamoyltransferase is a nuclear-encoded mitochondrial protein. Both the transcriptional regulation and the mitochondrial transport mechanisms of ornithine carbamoyltransferase are of interest. A cDNA library in which N. crassa DNA was inserted into the structural gene for beta-galactosidase was probed with antibodies to ornithine carbamoyltransferase. Two antibody-positive clones were chosen for further investigationn. These clones remained positive through several plaque screenings and so are quite stable. Protein extracts of cells infected with each recombinant phage contained a higher molecular weight beta-galactosidase band on SDS-polyacrylamide gels. Western blots of similar gels indicate that ornithine carbamoyltransferase antibodies react with the fusion protein product of each clone. The fusion proteins are larger by approximately 5 and 35 kD respectively. The subunit molecular weight of ornithine carbamoyltransferase is approximately 38 kD, so the latter clone may contain most of the ornithine carbamoyltransferase gene. Work is in progress to subclone the inserted DNA into an E. coli plasmid vector and to verify the identity of the cloned cDNA. The subcloned insert will be used to probe a genomic N. crassa DNA library in E. coli by colony hybridization.

1572 CLONING OF AUTONOMOUSLY REPLICATING SEQUENCES OF THE PENICILLIUM CHRYSOGENUM MITOCHONDRIAL DNA, Helmut Schwab[+], Ulf Stahl* and Karl Esser*, Biochemie Ges.m.b.H., A-6330 Kufstein-Schaftenau (+) and Institut für Allgemeine Botanik, Ruhruniversität D-4630 Bochum, FRG (*)

Mitochondrial (mt) DNA was isolated from a P.chrysogenum penicillin production strain. A detailed restriction map was established by analysing complete mt DNA and also cloned HindIII-fragments. Several HindIII and EcoRI fragments of the mtDNA were cloned into the vector pDAM1. This vector is not able to replicate in the yeast Saccharomyces cerevisiae. After transformation of S.cerevisiae with hybrids of pDAM1 and mtDNA fragments, three different fragments of P.chrysogenum mtDNA promoting autonomous replication in yeast could be identified. Experiments to analyse the replication ability of these fragments in P.chrysogenum are in progress.

1573 Genetic and Molecular characterisation of argB+ transformants of Aspergillus nidulans. A. Upshall, ZymoGenetics, Inc. 2121 N.35th Street, Seattle, WA 98103.

To obtain detailed information as to the nature of transformation events in filamentous fungi, thirty three argB- to argB+ (Berse et.al. Gene 25, 1983, 109-117.) transformants are being studied by both genetic and molecular means. Fourteen were obtained following transformation of a strain of the genotype biA1 methG argB with pMT201 (pBR329 carrying a 6kb fragment containing the argB+ sequence; provided by Prof. W. Timberlake); four following transformation of this same strain with pZM006 (pUC19 carrying a 3.3kb XbaI subclone of the BamHI fragment), and sixteen following transformation of a strain of the genotype pabaA1 yA2 argB with pZM006. To date:- 3 transformants have been shown to be diploid, heterozygous for arginine requirement; 10 show replacement of the argB- allele with the argB+ allele; in most of the remaining 23 transformants plasmid integration occurred at the chromosomal locus; one transformant has shown mitotic instability for 6 generations of single conidiospore propagation;selfed meioses of 28 transformants gave between 0 and 5% arginine auxotrophs, the remaining five, 12% or greater; in hybrid meioses with an argB+ strain, 8 transformants yielded argB- segregants at a frequency of 25% or greater. The results of the complete analysis will be posted at the meeting.

1574 THE amdS TRANSFORMATION SYSTEM FOR Aspergillus nidulans, [1]K. Wernars, [1]T. Goosen, [2]R.F.M. van Gorcom, [2]C.A.M.J.J. van den Hondel, [1]H.W.J. van den Broek, [1]Dept. of Genetics, Agricultural University, Wageningen, The Netherlands, [2]Medical Biological Laboratory TNO, Rijswijk, The Netherlands.

DNA-medicated genetic transformation of A. nidulans has been achieved now by incubating mycelial and conidial protoplasts from A. nidulans mutant strains with either plasmids, cosmids or linear fragments containing cloned structural genes (c.f. A. nidulans genes amdS/trpC/argB, Neurospora crassa gene pyr4, A. niger gene trpC). Each system seems to have its own pecularities with respect to transformation frequency, type of integration and stability of transformants.

We have studied the amdS transformation system in more detail. From the results we conclude that the amdS system has properties which are very useful for a general cloning system for Aspergillus. Specific aspects will be presented and discussed.

1575 ISOLATION AND ANALYSIS OF THE Aspergillus niger trpC GENE, [1]C.A.M.J.J. van den Hondel, [1]A. Kos, [2]K. Wernars, [2]H.W.J. van den Broek, [1]P.H. Pouwels, [1]Medical Biological Laboratory TNO, Rijswijk, The Netherlands, [2]Dept. of Genetics, Agricultural University, Wageningen, The Netherlands.

The Aspergillus niger trpC gene was isolated by complementation of E. coli trpC mutants. Subsequent transformation experiments of an A. nidulans trpC mutant as well as heterologous hybridisation experiments with both the A. nidulans trpC gene and the N. crassa trp-1 gene indicated that a complete, functional A. niger trpC gene was obtained. Finally the nucleotide sequence was determined.

The results of these analyses and a comparison of the structures of the A. niger trpC gene to that of A. nidulans and N. crassa will be presented.

1576 TRANSFORMATION OF PENICLLIUM CHRYSOGENUM, P. van Solingen, H.D. Muurling and B.P. Koekman, Gist-brocades N.V., P.O. Box 1, 2600 MA Delft, The Netherlands.

ABSTRACT
By complementation of an auxotrophic mutation, we have been able to transform P.chrysogenum with low frequency. The transforming plasmid pGB83, which also contains a piece of P.chrysogenum ribosomal DNA, becomes integrated into the Penicillium genome, as indicated by Southern blot analysis. In several instances, we succeeded in recovering pGB83, or its derivatives, from P.chrysogenum transformants by restriction endonuclease digestion of chromosomal DNA, ligation, and transformation to E.coli.

1577 MITOCHONDRIAL PLASMIDS FROM COCHLIOBOLUS HETEROSTROPHUS. Sally Van Wert, Robert Garber, Jim Oard, J. J. Lin, Gillian Turgeon, and Olen Yoder, Cornell University, Ithaca, NY 14583-0331.

We have recovered mitochondrial plasmids about 2 kb in size from two different isolates (T40 and T21) of Cochliobolus heterostrophus, a fungal pathogen of maize. The plasmids share homology but differ in restriction enzyme sites and in size by 100 bp. Both plasmids coexist with the intact mitochondrial chromosome and both map to the same unique location on the chromosome. There is one integrated copy of the plasmid sequence in the chromosome of every isolate we gave examined, although only two isolates contain the plasmid. The free and integrated forms of the plasmid are transcribed in vivo. The plasmid functions as an origin of DNA replication (ARS) in yeast.

Sequence analyses of mitochondrial plasmids from two other filamentous fungi (Podospora and Neurospora) have shown that in one case the plasmid is a Group II intron of oxi3 (Osiewacz and Esser, 1984) while in the other the plasmid is structually analagous to a Group I mitochondrial intron (Nargang et al., 1984). Sequencing of the two Cochliobolus plasmids and their integrated forms plus flanking sequences is in progress to determine if these plasmids are also derived from known mitochondrial genes.

1578 TRANSFORMATION OF ASPERGILLUS NIDULANS WITH AN OLIGOMYCIN-RESISTANT ALLELE OF THE MITOCHONDRIAL ATPSYNTHASE PROTEOLIPID GENE, Michael Ward and Geoffrey Turner, Bristol University, England, BS8 1TD

The proteolipid is a subunit of the F_0 portion of the ATPsynthase complex. Also known as the DCCD-binding protein, it is the site of action of oligomycin. Nuclear oligomycin-resistant (oliR) mutants have been isolated in A. nidulans and it has been inferred that these represent alterations in the proteolipid structural gene. Using a cDNA clone of the N. crassa proteolipid gene we have probed a phage gene bank and isolated the equivalent gene from an oliR strain of A. nidulans. Incorporation of this gene into various plasmid vectors has allowed it to be used in transformation studies and data on integration will be presented.The gene will confer oligomycin-resistance on a sensitive (wild-type) strain and can thus be used as a selectable marker without the need for auxotrophic mutations in the recipient strain. These experiments also confirm that the nuclear oliR mutations at the OLI C locus are indeed in the proteolipid gene.

The site of synthesis of the very hydrophobic proteolipid is cytoplasmic from where it must be transported to it's position of assembly in the inner membrane of mitochondria. In order to achieve this import the protein is first synthesised as a much larger preprotein with a 62 amino acid leader sequence. Sequencing the gene has allowed comparison of this region with that of the equivalent N. crassa gene and identified certain conserved regions which may be important for uptake into mitochondria and processing to the mature form.

1579 A COSMID FOR SELECTING GENES BY COMPLEMENTATION ASPERGILLUS NIDULANS: SELECTION OF THE DEVELOPMENTALLY REGULATED yA LOCUS, M. Melanie Yelton and William E. Timberlake, University of California, Davis, CA 95616.

We constructed a 9.9 kilobase cloning vector, designed pKBY2, for isolating genes by complementation of mutations in Aspergillus nidulans. pKBY2 contains the bacteriophage λ cos site, to permit in vitro assembly of phage particles; a bacterial origin of replication and genes for resistance to ampicillin and chloramphenicol to permit propagation in Escherichia coli; the A. nidulans $trpC^+$ gene to permit selection in Aspergillus; and a unique Bam HI restriction site to permit insertion of DNA fragments produced by digestion with restriction endonucleases Bam HI, Bgl II, Mbo I or Sau 3A. We used this cosmid to form a quasirandom recombinant DNA library containing 35-40 kilobase DNA fragments from a wild-type strain of A. nidulans. DNA from this library transformed a yellow-spored (yA^-), $pabaA^-$; $trpC^-$ Aspergillus strain (FGSC237) to $trpC^+$ at frequencies of approximately 10 transformants/μg DNA. Three of approximately 1,000 $trpC^+$; $pabaA^-$ colonies obtained were putative yA^+ transformants, because they produced wild-type (green) spores. DNA from each of the green-spored transformants contained pKBY2 sequences and DNA from two transformants transduced E. coli to ampicillin resistance following treatment in vitro with a λ packaging extract. The cosmids recovered in E. coli had similar restriction patterns and both yielded $trpC^+$ transformants of A. nidulans FGSC237, 85% of which produced green spores. Several lines of evidence indicate that the recovered cosmids contain a wild-type copy of the yA gene.

Metabolic Gene Regulation I

1580 REGULATION OF POLYAMINE SYNTHESIS IN NEUROSPORA, Rowland H.Davis, Department of Molecular Biology & Biochemistry, University of California, Irvine, Irvine CA 92717

Polyamines are indispensable to eukaryotes, and are rapidly synthesized at the onset of growth, and during neoplasia and differentiation. Ornithine decarboxylase (ODC) is a key enzyme of polyamine synthesis, rising greatly at the onset of growth and falling through inactivation of enzyme when it is no longer called for. The factors governing polyamine metabolic rates and the regulation of ODC in most organisms are poorly understood, owing to poor correlation of enzyme levels and polyamine pool sizes, and to lack of antisera to ODC. The pathway in Neurospora crassa resembles that found in mammals, and we have sought to determine the metabolic signal for, and the mechanism of variations in ODC activity. ODC activity varies in response to variations in the rate of spermidine synthesis (1), but not to variations of other intermediates of the pathway. The changes of the spermidine pool which accompany the rise and fall of ODC are quite small, in keeping with our demonstration that only 15% of the cellular polyamine pools is freely diffusible (2,3). Variations of ODC activity in response to spermidine are accompanied by corresponding changes in the level of ODC cross-reacting material detected with anti-ODC antiserum. The changes are not proportional: the loss of activity relative to loss of protein suggests that the enzyme may be inactivated by modification prior to its proteolytic removal.

Four partial mutants for ODC map to the spe-1 locus, defined by ODC-less mutants isolated previously (4). One has a 25-fold higher K_m for ornithine than normal and an increased thermostability; another is normal in these characteristics. All mutants either display cross-reacting material on Western blots or have residual ODC function. We conclude that spe-1 is the structural gene for the enzyme. The mutant strains offer materials with which to identify the cloned the gene by transformation and complementation.

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1581 Structure and Function of the NADP-specific Glutamate Dehydrogenase gene of Neurospora crassa. J.R.S. Fincham, Department of Genetics, University of Cambridge, U.K.

The structure of the N.crassa am gene was recently determined[1] and has the following interesting features:

(i) The transcription startpoints, of which there appear to be several within 20 base-pairs, occur 38-58 bp upstream of a TATAAA sequence. Studies are in progress to determine how much sequence upstream of the TATA box is required for transcription. At the transcription terminus there is no clear homologue of the higher eukaryote polyadenylation signal but, comparing the sequence with that at the 3' ends of at least one other Neurospora transcript[2] one finds a hint of a hitherto unrecorded consensus.

(ii) The gene contains two introns of 66 and 61 bases respectively. They do not contain the yeast internal consensus TACTAACA but, in common with all other introns recorded so far in filamentous fungi, they have a relaxed version of this consensus in the form CTPuAC. This loose consensus generally occurs 10-20 bases in from the 3' end of the intron and finds an echo in the somewhat inconstant consensus (CTGAC) found at the branch-point of the "laryat" intermediate in the splicing-out of introns of vertebrates[3,4].

(iii) The codon usage in am is strongly biassed in a manner characteristic of the "housekeeping" genes of Neurospora. The most striking bias is seen in the almost complete absence of codons with A in the third position. Recent work in my laboratory by P.A. Burns and J.A. Kinnaird (unpublished) has shown a double-frameshift revertant strain to have three sequential codons with third-position A in the frameshift region. This revertant shows a low level of glutamate dehydrogenase in extracts. It is not yet clear whether this is due to a reduced level of translation or to the increased thermostability of the mutant enzyme; further experiments are in progress. The general evidence relating to the possible effects of codon usage on level of gene expression will be briefly reviewed.

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1582 GENE ORGANIZATION AND REGULATION IN THE QUINIC ACID (QA) GENE CLUSTER OF NEUROSPORA, N. GILES, Department of Genetics, University of Georgia, Athens, GA 30602

The quinic acid gene cluster of Neurospora crassa, which controls the inducible utilization of quinic acid as a carbon source, has been cloned on a 18 kb segment of linkage group VII (1). Combined transformation and transcription experiments indicate that this cluster comprises seven genes transcribed as distinct mRNAs. Three of the genes (qa-2, qa-3, and qa-4) encode inducible qa enzymes while two other presumptive structural genes (qa-x and qa-y) have unknown functions (2). Genetic and molecular evidence indicates that there are two qa regulatory genes which encode, respectively, a repressor protein (qa-1S) and an activator protein (qa-1F) (3). Transcriptional evidence utilizing qa-1S and qa-1F mutants suggests that qa-1F acts positively in controlling transcription of itself (autogenous regulation) and of the other qa genes (Patel). The entire qa cluster has been sequenced and open reading frames identified for each of the seven gene products (Geever et al.). The two regulatory genes encode proteins of ca. 100,000 (repressor) and ca. 88,000 (activator) daltons (Huiet). S1 mapping data have identified mRNA initiation sites for each qa gene (Tyler). Several different repressor mutants have been cloned and sequenced (Huiet). These mutants contain single base pair substitutions in the qa-1S coding region. The function of the qa-1F gene product (activator) has been studied in several qa-2 activator-independent ($qa\text{-}2^{ai}$) mutants. Based on sequence data these mutants possess one of a variety of mutations consisting of short duplications, large DNA rearrangements, and "point" mutations, all in the 5' untranscribed region of qa-2 (4). Several of these mutations have been shown to exhibit certain characteristics of enhancer elements. The overall data suggest that two types of promoters occur in the qa gene cluster -- one type apparently requires only RNA polymerase II access while the second type also requires direct binding of activator (5). Studies on DNase I hypersensitive sites provide direct support for this hypothesis since the $qa\text{-}1F^{+}$ genotype is positively correlated with increased DNase I cleavage in the -200 to -88 region of qa-2, suggesting a direct interaction of the activator with chromatin in this region (6). Preliminary data obtained in transformation experiments utilizing truncated qa-1F donor DNA lacking all 5' untranscribed presumptive regulatory sequences (Case) suggest that regulation in the qa system involves, in part, interactions between the two regulatory proteins.

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1583 REGULATION OF NITROGEN METABOLISM IN NEUROSPORA, George A. Marzluf, Kimberly G. Perrine and Baek H. Nahm, Departments of Biochemistry and Genetics, The Ohio State University, Columbus, OH 43210

In Neurospora the expression of many enzymes of nitrogen metabolism is regulated by common genetic and metabolic signals (1). Thus, nitrate and nitrite reductase, xanthine dehydrogenase, uricase, allantoinase, L-amino acid oxidase, amino acid transport, and an extracellular protease as well as other enzymes are all subject to the control of the nitrogen regulatory circuit (1). Genetic signals of this circuit include the nit-2 gene, a major control gene required to turn on the expression of many unlinked nitrogen-related genes, and nit-4, which mediates nitrate induction of a subset of genes specific to nitrate metabolism. We have recently isolated and studied many nit-2 and nit-4 mutants as well as revertants for select mutants at each of these loci. One new mutant of nit-2 (allele KGP0220) has been demonstrated to be a nonsense mutation which can be suppressed by the unlinked suppressor SSu-1, known to be effective for UAG nonsense codons. It is intriguing that suppression does not occur in a heterokaryon in which the SSu-1 gene and the nit-2 nonsense mutation are in separate nuclei, but is efficient in a SSu-1 nit-2 homokaryon. The finding of a suppressible nonsense nit-2 mutant provides clear evidence that the product of this regulatory gene is a protein.

The enzyme uricase is present at a basal level in cells grown with abundant nitrogen and cannot be further repressed. Uricase activity increases about 5-fold when cells are limited for nitrogen and induced with uric acid. Using antibodies to immunoprecipitate uricase protein, we determined that uricase induction involves de novo enzyme synthesis and that the increase in enzyme activity depends upon a corresponding increase in the amount of the identical uricase species which is present at a basal level in fully repressed cells. Both poly(A)$^+$ RNA and total RNA isolated from repressed and from induced cultures have been translated in vitro to produce uricase of correct size. The results of these experiments suggest that regulation occurs at the level of transcription but also suggest that induced uricase mRNA differs from the uricase mRNA made in repressed cells.

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1584 REGULATION OF PYRIMIDINE METABOLISM IN NEUROSPORA, A Radford, F P Buxton and S F Newbury. Department of Genetics. University of Leeds, Leeds LS2 9JT, U.K..

The six steps in the pyrimidine biosynthetic pathway up to uridine-5'P are catalysed by enzymes encoded by five separate genes, the first two activities being specified by a single bifunctional gene. All pyrimidine auxotrophs respond to any normal pyrimidine base, nucleoside or nucleotide, but on good nitrogen sources, base uptake and supplementation is inefficient. Neurospora lacks thymidine kinase, so all supplied pyrimidines recycle through UMP: as a consequence, specific labelling of DNA is not possible.

In the biosynthetic pathway, the pyr-3 gene specifying the first two enzyme activities CPS and ACT is derepressed 4-5x by end product depletion, and fine control is via feedback on CPS. However, the regulatory mutant fdu-2 is insensitive to this repression. The third gene in the pathway, pyr-1 (DHO dehydrogenase) appears to be substrate-induced, but evidence of regulation elsewhere in the pathway is sparse.

Uptake of pyrimidine bases is by a mechanism involving uc-5, and nucleoside uptake is a property of the ud-1 locus. Both pyrimidine and purine nucleosides are taken up by the same transport system. Base and nucleoside uptake, although different systems, are both controlled by nitrogen metabolite repression and by the general nitrogen metabolite regulator gene nit-2. Repressed and derepressed levels of base uptake vary 90x, nucleoside uptake by 5x. Nucleoside uptake is also affected by the regulator gene fdu-2.

The salvage pathways for exogenous pyrimidine bases and nucleosides are known. Uridine, once taken up, is converted by the udk (uridine kinase) gene to UMP. Uracil is converted by uc-4 (phosphoribosyl transferase) to UMP. Deoxyribonucleosides are converted to ribonucleosides by uc-2 (2'-hydroxylase) and thymine to uracil by uc-3 (thymine 7-hydroxylase). The uc-1 gene appears to exercise regulatory control on several of these salvage steps, and udk is regulated by fdu-2.

There are thus several levels of regulation of pyrimidine metabolism, salvage regulated by uc-1, aspects of biosynthesis, uptake and salvage by fdu-2, and pyrimidine metabolism in general by nitrogen metabolite repression via nit-2.

The pyr-4 (OMP decarboxylase) has been cloned by complementation of E. coli pyrF, and also complements Saccharomyces and Aspergillus. The Saccharomyces equivalent clone functions in E. coli, but is not expressed in Neurospora. Because of these differences in expression of the equivalent Neurospora and Yeast clones, sequence comparisons are of interest. The Yeast ura3 sequence is known, pyr-4 is being determined, and the two will be compared.

Metabolic Gene Regulation II

1585 REGULATION OF GENE EXPRESSION IN ASPERGILLUS NIDULANS, Herbert N. Arst, Jr. Department of Genetics, University of Newcastle Upon Tyne NE1 7RU, England.

The ascomyete Aspergillus nidulans is a particularly favourable eukaryote for classical and molecular studies of the regulation of gene expression. Control of gene expression in A.nidulans occurs mainly at transcription and is mediated by proteins encoded by regulatory genes which can be classified as pathway-specific, wide domain or integrator. Pathway-specific regulatory genes mediate induction or repression by a metabolite of the pathway and most of those identified are positive-acting. The probable product of one (uaY) has been isolated. For another (nirA) two distinct kinds of altered function alleles have been selected in addition to those resulting in loss of function. Wide domain regulatory genes mediate responses to a nutritional sufficiency or environmental condition affecting many pathways. The best characterised of these, areA, mediates nitrogen metabolite repression, ensuring preferential utilisation of favoured nitrogen sources (the actual effector probably being L-glutamine). In addition to loss of function alleles, two categories of mutations resulting in nitrogen metabolite derepression have been obtained, altering either the structure or the quantity of the areA protein. A pseudogene, areB, can substitute for areA when activated by certain chromosomal rearrangements. Preliminary evidence suggests that areA might mediate oxygen repression as well as nitrogen metabolite repression, at least of some activities. Carbon catabolite repression is mediated by a negative-acting wide domain regulatory gene creA. Other wide domain regulatory genes include positive-acting palcA, mediating phosphate repression of activities involved in phosphorous acquisition, and, possibly, negative-acting suAmeth mediating L-cysteine repression of activities involved in cysteine biosynthesis and perhaps more generally in sulphur acquisition. Another form of wide domain regulation involves the regulation of certain permeases and secreted enzymes whose synthesis is dependent on the pH of the growth medium. For example, alkaline but not acid phosphatases are synthesised in alkaline media, the reverse being true in acidic media. Mutations have identified, in addition to a putative wide domain regulatory gene mediating pH regulation, several genes probably involved in the synthesis of a small effector molecule. Integrator genes are positive-acting regulatory genes mediating concomitant induction of activities encoded by two or more non-contiguous structural genes of which at least one can also be expressed independently by an alternative induction mechanism. In the presence of ω-amino acids, the intA product integrates the expression of amdS (structural gene for acetamidase) with that of three genes involved in ω-amino acid utilisation, whereas in other contexts (e.g. the presence of acetate or benzoate) amdS can be expressed independently. In some cases mutations have also identified receptor sites for regulatory gene products. Included amongst cis-acting regulatory mutations are chromosomal rearrangements fusing structural genes to new regulatory regions. (References are too numerous to list here but are contained in my review in Microbiological Sciences 1, 137-141 (1984)).

1586 STRUCTURE AND EXPRESSION OF THE ASPERGILLUS *amd*S GENE, Michael J. Hynes, Joan M. Kelly, Catherine M. Corrick and Timothy J. Littlejohn, Department of Genetics, University of Melbourne, Parkville, Victoria 3052, Australia.

The *amd*S gene codes for an amidase enzyme allowing utilization of acetamide. *Cis* and *trans* acting mutations affecting regulation have been isolated and used to show that the gene is subject to control by multiple independently acting regulatory genes. The wildtype gene (1) and some of the mutant genes have been cloned and sequenced. Evidence for a *cis*-acting control region 3' to the start point for transcription will be presented. Transformation studies using the *amd*S gene have shown that it is possible to generate transformants with at least 100 copies of the gene. This results in apparent titration of the *amd*R gene product leading to reduced growth on substrates under *amd*R control. Using this assay it has been possible to show that this results from a sequence at the 5' end of the gene. Multiple copies of an *amd*I9 containing sequence but not an *amd*I$^+$ sequence lead to reduced growth on acetate. The *amd*I9 mutation is due to a single base pair change in the 5' upstream region and the results indicate that this leads to increased affinity for the *fac*B regulatory gene product. The *amd*S gene has been used to transform *A. niger* to generate multiple gene copies leading to high *amd*S expression levels.

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1587 MOLECULAR ANALYSIS OF ALCOHOL METABOLISM GENES IN ASPERGILLUS, J.A. Pateman, C.H. Doy, J.E. Olsen, H.J. Kane and E.H. Creaser, Department of Genetics and Centre for Recombinant DNA Research, Research School of Biological Sciences, Australian National University, Canberra, ACT, Australia.

A single alcohol dehydrogenase (ADH) and a single aldehyde dehydrogenase (AldH) are responsible for the utilisation of ethanol as a carbon source in Aspergillus nidulans. Both enzymes are induced by ethanol and/or acetaldehyde and subject to carbon catabolite repression. ADH is coded by a gene alcA on chromosome VII and AldDH is coded by a gene aldA on VIII. A regulatory gene alcR is closely linked to alcA and acts via a positive-acting regulatory protein to regulate the transcription of alcA and aldA (1). The deletion mutant alcA55 alcR3 lacks both the alcA and alcR genes and was shown by Southern blots to lack a 3.8kb BamHI fragment present in the wild-type. The 3.8kb BamHI fragment was subcloned into pBR322 and a clone pAN4-8 used to screen an EMBL3A A. nidulans genomic bank. After purification three overlapping clones 4-K-1, 4-K-3 and 4-K-4 covering about 24kb were obtained and their restriction enzyme sites mapped. The clones were used to probe Southern blots of some alcA and alcR mutants thought from genetic analysis to be deletions. The mutants alcA51, alcA83 and alcR54 gave different restriction fragment patterns to the wild-type which located the alcA and alcR genes in certain restriction fragments. The restriction analysis also indicated that all three mutants had additional DNA inserted at the mutation sites. Southern blot analysis of the 4K clones using cDNA probes made from induced and repressed mRNA indicated a transcribed region, which is larger than the 1.4kb needed to code for ADH. A probe containing the Saccharomyces cerevisiae gene ADC1, which codes for ADH1, hybridised weakly to a region similar but not identical to the transcribed region of the 4K clones. DNA sequencing of the whole alcA-alcR region is under way and the current data and interpretation will be presented at the meeting.

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1588 GENE CLUSTERS AND THEIR REGULATION IN ASPERGILLUS NIDULANS, Claudio Scazzocchio, Department of Biology, University of Essex, U.K. Colchester, Essex and Institut de Microbiologie, Universite Paris-Sud, Orsay, France.

Genes coding for enzymes catalising related metabolic steps may show different degrees of clustering in the filamentous fungi. I should discuss mainly two systems of Aspergillus nidulans.

The ethanol utilisation regulon is comprised by the structural gene for alcohol dehydrogenase I (alcA), the structural gene for aldehyde dehydrogenase (aldA) and their cognate positive control gene, (alcR)(1). The alcA and alcR genes are closely linked but unlinked to aldA. The expression of alcA and aldA is induced by ethanol and repressed by glucose, while a second alcohol dehydrogenase (ADHII) is repressed by both glucose and ethanol. The evidence is consistent with alcR acting as a positive control gene for alcA and aldA, but as a negative control gene for the gene coding for ADHII(2).

The proline utilisation cluster prnADBC, comprises the positive regulatory gene prnA, the genes coding for the first and second enzyme of the pathway, (prnD and prnC) and a gene coding for a proline permease (prnB)(3). The expression of the structural genes is subject to specific induction and to both carbon and nitrogen metabolite repression.

I shall describe a brute force method that has been specially useful in isolating clones involving gene clusters. By this method we have obtained both alcAalcR and prnADBC genomic clones. alcA and aldA had been also isolated previously by conventional cDNA cloning and these used to isolate alcAalcR and aldA genomic clones (4). We have used transformation and complementation of well characterised mutations, and specially with the prn cluster, restriction mapping of genetically characterised deletions, to establish the position of individual genes within the genomic clones.

Northern blotting is consistent with specific induction of all structural genes being at the transcription level in both systems. Northern blotting has also revealed an additional transcript between prnA and prnD in the prn cluster. All deletions ending in prnB show a polar effect on prnC (3). Our results suggest that this effect is due to the deletion of a sequence within prnB or its cognate cis-acting region necessary for high expression of prnC rather than to a dicistroninic prnB-prnC message as previously thought (3).

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Metabolic Gene Regulation

1589 GENETIC CONTROL OF CHROMATIN STRUCTURE 5' TO THE QA-X AND QA-2 GENES OF NEUROSPORA, James A. Baum and Norman H. Giles, University of Georgia, Athens, GA 30602

The roles of the qa-1F and qa-1S regulatory genes of Neurospora in modifying the chromatin structure of two qa structural genes have been studied by mapping DNase I hypersensitive sites in qa chromatin isolated from wild-type, qa-1F^{-} (non-inducible) mutants, qa-1S^{c} (constitutive) mutants, qa-1S^{-} (non-inducible) mutants, and from activator protein-independent mutants of qa-2 (qa-2^{al}). DNase I hypersensitive sites in the 5' region of the qa-x and qa-2 structural genes increase in number and sensitivity upon induction of transcription with quinic acid. Both qa-1F^{-} and qa-1S^{c} mutations are associated with alterations in the DNase I sensitivity of the qa-x and qa-2 region, the latter mutations resulting in the common 5' flanking region of these genes being accessible to DNase I. The qa-1F^{+} genotype is correlated with increased DNase I cleavage in the -200 to -88 region of qa-2, a region previously implicated in qa-1F regulation of RNA polymerase II access to the qa-2 promoters. DNase I and exonuclease III digestion of qa-2 chromatin suggests that a 20-30 bp sequence within this region may be involved in transcriptional regulation of qa-2. This nuclease protected region corresponds closely to a sequence with imperfect dyad symmetry (C G G G T A A T C|G C T T A T C C G) centered at position -129.

1590 CLONING AND DNA SEQUENCE ANALYSIS OF THE 3-PHOSPHOGLYCERATE KINASE GENE FROM ASPERGILLUS NIDULANS, John M. Clements and Clive F. Roberts, University of Leicester, Leicester LE1 7RH, England

The 3-phosphoglycerate kinase gene (PGK) from Aspergillus nidulans has been cloned from a λL47 genomic library using the equivalent yeast gene as a hybridization probe. The complete nucleotide sequence of the gene including the 5' and 3' flanking sequences has been determined. In contrast to the yeast gene, the Aspergillus PGK gene contains two 57 base pair introns. The structure and features of the PGK gene will be fully discussed and data on the expression of PGK presented.

1591 BIOCHEMICAL GENETICS OF NEUROSPORA PYRUVATE KINASE: AN ANALYSIS OF GENES AND MESSAGES, M. Devchand and M. Kapoor, University of Calgary, Calgary, Alberta, Canada, T2N 1N4

Pyruvate kinase (PK) of N. crassa, an allosteric, enzyme is being used as a model system for studying the genetic regulation of constitutive enzymes. The polysomal fraction from 16-hour old cultures was immunoprecipitated with anti-PK antibody raised in rabbits. RNA isolated from these polysomes was translated in vitro using either the Wheat Germ and Rabbit Reticulocyte Lysate or a lysate prepared from wild-type Neurospora mycelium. Analysis of the translation products showed this RNA fraction to be highly enriched in PK-specific mRNA. Fractionation on oligo(dT)cellulose, yielding poly(A^{+}) and poly(A^{-}) fractions, and in vitro translation followed by analysis of the products on immuno-affinity columns, showed the presence of PK-specific mRNA in both fractions. Northern blots of RNA fractions hybridized to a nick-translated, [^{32}P]-labelled, DNA fragment from the yeast PK gene cloned into the plasmid YEp13 also revealed two distinct mRNA species. This was further confirmed by dot blot hybridization of fractions of immunoprecipitated polysomal RNA, subjected to centrifugation on a 10-40% sucrose density gradient. Northern hybridization and dot blots indicated that one mRNA species corresponded to the poly(A^{+}) and the other to the poly(A^{-}) fraction. Experiments with S1 nuclease digestion using the above-mentioned probe with both mRNA species showed a single protected fragment. Southern blots of Neurospora genomic DNA digested with a number of restriction endonucleases showed, in each case, the hybridization of at least two bands with the same probe. Genomic libraries of N. crassa are currently being screened for clones representing the pyruvate kinase genes.

1592 GENETIC AND MOLECULAR ORGANIZATION OF THE QUINIC ACID (qut) GENE CLUSTER IN Aspergillus nidulans Francisco da Silva, A.J.[1]; Roberts, C.F.[1]; Hawkins, A.R.[2]. 1. Dept. of Genetics, University of Leicester, Leicester LE1 7RH. U.K. 2. Dept. of Genetics, Ridley Building, University of Newcastle-upon-Tyne, NE1 7RH. U.K.

Quinic acid can be used as an alternative carbon source by A.nidulans. The first three enzymes in the biochemical pathway are highly induced upon expression of the structural genes; the system is also subject to carbon catabolite repression.

Genetic and biochemical analysis of quinic acid non-utilizing (qut$^-$) mutants in haploid and heterozygous diploid strains, reveals that the three structural genes are tightly linked and that they are under genetic regulation by two nearby closely linked control genes. One of these has the properties of a repressor gene, inhibiting the expression of the second control gene, which acts as an activator. In the presence of the inducer, quinic acid, the activator gene is transcribed and enables expression of the structural genes. Furthermore, certain observations suggest that in addition to quinic acid the products of the two control genes together interact in promoting transcription of the structural genes.

To analyse this system at the molecular level, the gene cluster has been cloned using DNA fragments of the equivalent genes in Neurospora crassa as hybridization probes. The DNA sequence of the promoter regions of the three structural genes, are currently being determined, and will also be discussed.

1593 REGULATION OF THE β-1,4-GLUCOSIDASE PRODUCED BY CANDIDA WICKERHAMII, Shelby N. Freer, Northern Regional Research Center, 1815 N. University St., Peoria, Il. 61604

Candida wickerhamii NRRL Y-2563 produced a constitutive β-glucosidase (3-8 U/ml) when grown aerobically in complex media containing either glycerol, succinate, xylose, or cellobiose as the carbon source. The addition of high concentrations of glucose (>75 g/liter) repressed β-glucosidase production (<0.3 U/ml), however, this yeast did produce β-glucosidase when the initial glucose concentration was less than or equal to 50 g per liter. When grown aerobically in media containing glucose plus the above carbon sources, diauxic utilization of the carbon sources was observed and the expression of β-glucosidase was glucose repressed. Surprisingly, anaerobiosis derepressed the glucose repression of β-glucosidase. When grown anaerobically in media containing 100 g of glucose per liter, C. wickerhamii produced 6-9 U/ml enzyme activity and did not demonstrate diauxic utilization of glucose-cellobiose mixtures. To my knowledge, this is the first report of derepression of a glucose repressed enzyme by anaerobiosis.

1594 Sequence of the 5' upstream regions of alc A and ald A structural genes in Aspergillus nidulans. David I. Gwynne, Mark H. Pickett and R. Wayne Davies, Allelix Inc., 6850 Goreway Drive, Mississauga, Canada, and Claudio Scazzocchio, Deptartment of Biology, University of Essex, U.K.

alc A and ald A are the structural genes which encode alcohol dehydrogenase I and aldehyde dehydrogenase in Aspergillus nidulans. Both alc A and ald A and a regulatory gene alc R are involved in ethanol utilization in this ascomycete. The regulation of both structural genes is under common control and involves the positive regulator, alc R and the negative regulatory gene cre A, responsible for carbon catabolite repression. alc A and ald A are inducible by ethanol, acetaldehyde and threonine and both transcripts represent a significant fraction of the mRNA mass under induced conditions. It was felt that a comparison of the cis acting regulatory regions of alc A and alc R would show common sequence elements representing common control regions. Therefore, both the alc A and ald A coding regions and flanking DNA have been cloned and the 5' upstream sequences have been compared.

1595 Cloning The Gene For The Neurospora crassa Plasma Membrane ATPase, Karl M. Hager and Carolyn W. Slayman. Yale University, New Haven, CT 06511

The plasma membrane of Neurospora crassa contains a proton translocating ATPase which couples the energy of ATP hydrolysis to the generation of an electrochemical gradient of protons across the membrane. In turn the proton gradient drives the transport of sugars and amino acids into the cell. The ATPase consists of a single M_r = 104,000 polypeptide subunit, which has been purified by preparative SDS gel electrophoresis. Polyclonal rabbit antibody was raised against this polypeptide and shown to be monospecific by Western blotting and immunoprecipitation. When the antibody was used to screen a Neurospora λgt11-cDNA expression library, 12 clones were isolated falling into three distinct groups on the basis of size (500bp, 350bp, 225bp). Experiments are now in progress to characterize the clones.

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1596 CHARACTERIZATION OF QA-1 REGULATORY MUTATIONS BY DNA SEQUENCE ANALYSIS
Layne Huiet & Norman H. Giles, University of Georgia, Athens, GA 30602

The qa (quinic acid) gene cluster of Neurospora crassa is controlled by two regulatory genes, qa-1S and qa-1F. These two genes control their own transcription and that of the five qa structural genes. Qa-1F$^-$ mutants are noninducible and recessive, and revert to wild-type. Uninducible qa-1S mutants (qa-1S$^-$) however are semidomiant and revert to constitutivity, suggesting that qa-1S has a negative regulatory function. In order to further investigate the regulatory role of qa-1S in qa gene expression, qa-1S was cloned from several qa-1S noninducible and qa-1^c constitutive mutants and the mutation(s) localized by nucleotide sequencing. The qa-1S$^-$ mutations occurred within the C terminus of the coding region previously assigned to the qa-1S gene. However, the mutations fell outside the 5' terminal sub-region of qa-1S required to transform the corresponding Neurospora mutants, suggesting that transformation of qa-1S$^-$ mutants occurs by gene disruption. The constitutive qa-1 mutations were also localized to qa-1S. These two observations strongly support a negative regulatory role for qa-1S.

1597 CLONING AND REGULATION OF NITRATE-INDUCIBLE GENES IN NEUROSPORA CRASSA
Barry K. Hurlburt and Reginald H. Garrett, University of Virginia Charlottesville, Va. 22901

Nitrate assimilation in Neurospora crassa represents a dramatic example of the genetic regulation of metabolic potentiality in lower eukaryotes. Nitrate induction of this pathway effects a 500-fold increase in the assimilatory enzymes, nitrate reductase and nitrite reductase, over the levels found in glutamine-grown mycelia. Expression of the structural genes for these enzymes, nit-3 and nit-6 respectively, requires the positive action of two regulatory genes, nit-2 and nit-4. In order to understand the molecular interactions underlying this regulation, the cloning of nitrate-inducible genes has been undertaken through application of a differential hybridization strategy.

Reverse transcription of poly(A$^+$) RNA isolated from nitrate-induced and glutamine-repressed mycelia, respectively, generates "plus" and "minus" cDNA probes. These probes have been hybridized to a Pst-1 derived genomic library of N. crassa DNA in pBR322. Eight nitrate-inducible DNA sequences were identified through specific hybridization to the nitrate-induced ("plus") cDNA pool only, and three sequences gave selective hybridization with the glutamine-repressed ("minus") cDNA pool. These eleven clones are being used as probes in hybridizations against RNA isolated from wild type and nit mutant mycelia grown under different nutritional regimens in order to correlate the clones with known nit genes and to gain insight into the genetic regulation of nitrate assimilation.

1598 AN ANALYSIS OF THE HEAT SHOCK GENES AND PROTEINS OF NEUROSPORA CRASSA, M. Kapoor, Department of Biology, The University of Calgary, Calgary, Alberta, Canada, T2N 1N4

Exposure to hyperthermic conditions elicits a rapid induction of synthesis of a set of heat shock proteins (hsps), with the concomitant suppression of synthesis of some normal cellular proteins. Neurospora mycelium, grown at normal temperature for 12 to 14 hours and subjected to heat shock at 48°C, was pulse-labelled with [^{35}S]methionine. SDS-PAGE analysis of cell extracts exhibited 9 polypeptides, labelled specifically, under heat stress (nhsps). The labelled hsps were analyzed by 2-dimensional immunoelectrophoresis (CIE). Antisera against normal and shocked cell extracts permitted the resolution of ~ 35 immunoprecipitates in normal cells, and in shocked cells identified new polypeptides synthesized upon heat shock as well as those exhibiting an enhanced production. Stained bands corresponding to nhsp70 and nhsp80 were excised from the gels and polyclonal antibodies raised against the eluted proteins enabled the identification of their respective immunoprecipitates. For a genetic analysis Southern blots of N. crassa genomic DNA, restricted and transferred to nitrocellulose paper and blots of RNA from normal and shocked cells were hybridized to a nick-translated fragment of the coding region of the hsp70 gene of Drosophila. A Neurospora genomic library was explored for sequences hybridizing with this probe. The induction of the synthesis of nhsps was monitored using immunological and molecular hybridization techniques.

1599 HEAT SHOCK UNCOUPLES TUBULIN EXPRESSION FROM MITOSIS IN PHYSARUM, Thomas G. Laffler and John J. Carrino, Northwestern University, Chicago, IL 60611.

In the myxomyceyte Physarum polycephalum, tubulin synthesis is subject to mitotic cycle control. Virtually all tubulin synthesis is limited to a two hour period immediately preceding mitosis, and the peak of tubulin protein synthesis is accompanied by a parallel increase in the level of tubulin messenger RNA. The mechanism by which the accumulation of tubulin mRNA is turned on and off is not clear. To probe the relationship between tubulin regulation and cell cycle controls, we have used heat shocks to delay mitosis, following the pattern of tubulin synthesis during these delays. Two peaks of tubulin synthesis are observed after a heat shock. One occurs at a time when synthesis would have occured without a heat shock, and a second peak immediately precedes the eventual delayed mitosis. These results are clearly due to altered cell cycle regulation. No mitotic activity is detected in delayed plasmodia at the time of the control mitosis, and tubulin behavior is shown to be clearly distinct from that of heat shock proteins. We believe that the tubulin family of proteins are subject to regulation by a thermolabile mitotic control mechanism, but that once the cell has been committed to a round of tubulin synthesis the "tubulin clock" runs independently of the heat sensitive system. In delayed plasmodia, the second peak of synthesis may be turned on by a repeat of the committment event.

1600 CLONING AND ANALYSIS OF THE HIS-3 GENE OF NEUROSPORA CRASSA, Timothy L. Legerton and Charles Yanofsky, Stanford University, Stanford, CA 94305.

The his-3 gene of N. crassa, which encodes a trifunctional polypeptide of histidine biosynthesis, was cloned by complementation of an E. coli histidine auxotroph using a plasmid pool of N. crassa DNA. The gene was sequenced and found to have extensive amino acid sequence homology with the his-4 gene of S. cereviseae. The C-terminal portion of the polypeptide encoded by his-3 shows amino acid homology with the his-D gene of S. typhimurium.

Northern analysis using the cloned his-3 gene as a probe identified a 2.7 kb transcript. The level of this transcript increased in cultures of N. crassa treated with 3-aminotriazole, which has been shown to cause cross-pathway derepression of enzymes in a number of amino acid biosynthetic pathways in N. crassa. This observation is consistent with the hypothesis that cross-pathway regulation of his-3 occurs at the level of transcription.

The intact gene and clones bearing deletions at the 5' end of the gene have been used to transform a his-3 mutant of N. crassa. The transformants are being analyzed to identify sequence elements involved in the regulation of his-3 expression in N. crassa.

1601 POLYMORPHISM IN THE EXTRANUCLEAR MUTANT, [mi-3], OF NEUROSPORA CRASSA, E.G. Lemire and F.E. Nargang, Genetics Department, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

The Neurospora crassa extranuclear mutant [mi-3] is characterized by slow-growth and a deficiency in cytochrome c oxidase levels. Though the actual lesion leading to the [mi-3] phenotype remains enigmatic, it is known that the mutant produces a larger cytochrome oxidase subunit I polypeptide. The mutant polypeptide represents the unprocessed precursor of the mature wildtype subunit I.

We have now located a restriction fragment length polymorphism in [mi-3] mitochondrial DNA (mt DNA) relative to wildtype mtDNA by electrophoresis of RsaI digested mtDNA on polyacrylamide gradient gels. Restriction mapping has localized this polymorphism to a position just upstream from the subunit I encoding oxi-3 gene. DNA sequencing and genetic analysis are underway to determine whether or not this polymorphism is related to the [mi-3] lesion.

1602 *gdh*, A NITROGEN CATABOLISM REGULATORY LOCUS IN *GIBBERELLA ZEAE*? John F. Leslie, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506

gdh was isolated as a nitrate non-utilizing mutant of *Gibberella zeae* (*Fusarium roseum* "Graminearum"). This mutant can grow only when one of the amino acids arginine, aspartic acid, asparagine, citrulline, glutamic acid, glutamine, histidine, ornithine, phenylalanine, proline, or tyrosine is available as a nitrogen source. NADPH-dependent glutamate dehydrogenase activity levels in both mutant and parental strains are comparable when the cells are grown in a medium containing glutamine or monosodium glutamate as a sole nitrogen source. The effective metabolism of glutamate to glutamine and glutamine to glutamate suggests that both functional glutamine synthetase and glutamate synthase systems are present. The possibility that the *gdh* mutant strain is impaired in the transport of both inorganic and organic nitrogen vs. the possibility that the mutant is defective in general regulation of nitrogen catabolism cannot yet be separated. The latter possibility seems preferable however, since the *gdh* mutant can utilize only a few amino acids as sole nitrogen sources while the parent can use NO_3, NO_2, NH_4, urea, formamide, β-alanine, and most purines and amino acids as sole nitrogen sources; a common transport mechanism for such a diverse group of compounds seems unlikely.

1603 MOLECULAR CHARACTERIZATION OF AN ASPERGILLUS NIDULANS ALCOHOL DEHYDROGENASE GENE. Gary L. McKnight, Hiroyuki Kato, Marilyn D. Parker and Alan Upshall, ZymoGenetics, Inc., Seattle, WA 98103.

We have isolated a 1350 bp cDNA, derived from Aspergillus nidulans poly (A)+ RNA, which encodes a functional alcohol dehydrogenase. The cDNA codes for a protein containing 352 amino acids, which displays approximately 50% homology to the alcohol dehydrogenases of S. cerevisiae and S. pombe. The corresponding genomic DNA fragment has been isolated and shown to contain a putative intron in the coding region of the gene. A comparison of the cDNA and genomic DNA sequences will be presented. Based on Northern blots, the mRNA level of this gene is approximately 10-20 fold higher in cells grown in the presence of 1% ethanol and is not decreased by the additional presence of 1% glucose. These results are being confirmed by the use of a gene fusion with lacZ.

Experiments currently in progress include: 1) a determination of the ability of S.cerevisiae to utilize the A. nidulans promoter and to process the putative intron; and 2) an analysis of the promoter efficiency in Aspergillus species.

Molecular Genetics of the Cytoskeleton

1604 FLAGELLAR SIZE CONTROL IN CHLAMYDOMONAS, Jonathan W. Jarvik and Michael R. Kuchka, Carnegie-Mellon University, Pittsburgh, PA 15213

We are pursuing a genetic and biochemical analysis of flagellar size control in Chlamydomonas reinhardtii. The problem has two rather different aspects - one molecular biological and one cell biological. From a molecular biological perspective, flagellar size control can be seen as a problem of axoneme elongation - and therefore, probably, of microtubule assembly. From a cell biological perspective, it can be seen in terms of organelle size regulation - especially in view of the fact that the flagellum is a distinct membrane bounded cellular compartment. We have identified three genes - shf-1, shf-2 and shf-3 - in which mutations with short flagella phenotypes exist. The short flagella appear functionally normal, with the single exception that their length is regulated to an abnormal value. Using these and other mutants, a number of in vivo length-adjustment experiments have been performed. Results argue against several otherwise attractive models for the cellular basis of flagellar size control (1,2).

Certain shf,shf double mutants are flagellaless, and therefore immotile, and so it is possible to select Shf^+ revertants in those backgrounds. Using such selections, we have identified both dominant and recessive suppressors of shf-1 and shf-2 mutations. Gel electrophoretic analyses of flagellar proteins from mutant and suppressor-carrying strains have identified several proteins which may be involved in the size control process.

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1605 IDENTIFICATION AND FUNCTION OF B-TUBULIN GENES IN ASPERGILLUS by G. May, Ph.D., J. Weatherbee, Ph.D., and N. R. Morris, M.D., Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854.

2-D gel analysis of Aspergillus tubulin suggests two B-tubulin genes, benA coding for two peptides, B1 and B2, and tubC, coding for B3 (Weatherbee & Morris, J. Biol. Chem., in press). Two B-tubulin clones have been isolated from a genomic library and verified as B-tubulin by partial sequencing. These were subcloned into an integrative transforming vector containing the Neurospora $pyr4^+$ gene and used to transform a uridine-requiring $pyrF^-$ strain of Aspergillus to uridine prototrophy. DNA blot analysis of transformants revealed that the plasmids integrated specifically. One clone, B5, mapped to the benA locus, while the other, B14, mapped elsewhere. A plasmid (pGM6) carrying an internal fragment of the B14 sequence was used to disrupt the gene by integrative transformation. Transformation of benA22 with pGM6 resulted in the disappearance of the B3 spot. Thus the B14 sequence represents tubC. BenA mutants grow in the presence of benomyl but fail to conidiate. After disruption of tubC conidiation became resistant to inhibition by benomyl. We conclude that B3 participates in conidiation but is unimportant for vegetative growth.

1606 THE MOLECULAR BIOLOGY OF MICROTUBULES IN ASPERGILLUS, Berl R. Oakley, Department of Microbiology, The Ohio State University, Columbus, OH 43210

We are attempting to determine the mechanisms of microtubule-mediated motility by isolating mutations that affect microtubule function and characterizing these mutations genetically, morphologically and biochemically. We have isolated more than 100 cold-sensitive (cs-) revertants of benA33, a heat-sensitive (hs-) β-tubulin mutation that blocks the functioning of microtubules at high temperatures (1). Of 78 cs- revertants analysed genetically, reversion was due to mutations closely linked to benA33 in 44 cases and to mutations unlinked to benA33 in 34 cases. We have mapped 18 of the closely linked revertants percisely with respect to benA33. All map to the right of benA33 and the recombination frequencies with respect to benA33 range from 0.000028 to 0.00012. The close linkage of these mutations to benA33 argues that they are in the benA gene. Of the remaining 34 revertants, 31 have been analysed. In 10 revertants, cold sensitivity is caused by mutations that coincidentally occcurred during mutagenesis and that have nothing to do with the suppression of benA33. In the remaining 21 cases, cold sensitivity is caused by mutations that cause the suppression of the heat sensitivity conferred by benA33. Of the 21 cs- suppressors of benA33, 16 map to the tubA, α-tubulin, gene and four map elsewhere. Nuclear division and migration are inhibited in many of the revertants at a restrictive temperature, indicating that microtubule functioning is disrupted. Mitochondrial movement is not inhibited, however, indicating that nuclei and mitochondria may move by different mechanisms. We have found that the frequency of non-disjunction is significantly increased in diploids carrying benA33 and in diploids constructed from some of the benA33 revertants. In an effort to identify the non-tubA suppressors of benA33, we have developed improved transformation frequencies and are now attempting to clone these suppressors by complementation. In summary, we have isolated many cs- α- and β-tubulin mutations as revertants of benA33. These mutations inhibit microtubule functioning and sequence analysis of these mutations should allow us to define some of the essential regions of the tubulin molecules. The identification of the non-tubA suppressors of benA33 should allow us to identify non-tubulin proteins that are involved in the functioning of microtubules. Supported by NIH grant GM31823.

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1607 GENE STRUCTURE AND REGULATION OF GENE EXPRESSION IN DICTYOSTELIUM R.A. Firtel, T. Crowley, S. Datta, R. Gomer, S. Mann, M. Mehdy, W. Nellen, and C. Reymond, Department of Biology, University of California San Diego, La Jolla, California 92093

We have been interested in the mechanisms of differential control of gene expression in Dictyostelium at the cellular and molecular levels. We have identified coordinately regulated gene sets which are expressed at various times during the developmental cycle. One set of genes, designated preaggregation genes, is induced by pulses of cyclic AMP which mimic the endogenous cyclic AMP pulsing during aggregation. These genes are repressed by high constant levels of cyclic AMP. Discoidin I genes comprise a three-member coordinately regulated multigene family which is induced upon the initiation of starvation and is repressed by cyclic AMP. We have also examined the regulation of cell-type-specific genes, those preferentially expressed in either prestalk or prespore cells. We have shown that cyclic AMP and another factor, CMF, are required for the induction of both sets of genes. Both classes require continuous cyclic AMP for the maintenance as well as the induction of gene expression. In addition, a cell-surface interaction is necessary for the expression of prespore mRNAs. We have also identified other conditions which can be used to distinguish between the regulation of these two classes of genes, indicating that prespore and prestalk genes have cell-type-specific as well as shared regulatory mechanisms. We have shown that the Dictyostelium ras gene is expressed in vegetative cells and also later in development specifically in prestalk cells, and that the mode of regulation of the Dictyostelium ras gene by cyclic AMP and other factors is the same as other prestalk genes. Lastly, we have been investigating the 17-20-member multigene family encoding actin.

We have been using DNA-mediated transformation to examine the regulatory regions controlling the expression of these genes. Genes transformed back into Dictyostelium appear to be regulated properly. For example, the Dictyostelium ras gene shows the same regulation by cAMP as the endogenous ras gene and other prestalk genes. In vitro mutagenesis experiments are being performed to identify cis-acting sequences necessary for the proper regulation of these coordinately regulated families. To complement these studies, antibodies against proteins encoded by the cell-type-specific genes are being used to examine the spatial distribution of the expression of these genes in the developing aggregate. We expect that understanding how the prestalk and prespore genes are regulated will give us insight into the decision-making process of cells entering either the prestalk or the prespore pathway.

Organization and Evolution of Genomes

1608 OOMYCETE MITOCHONDRIAL GENOMES, Michael E. S. Hudspeth, Div. of Biological Sci., University of Michigan, Ann Arbor, MI 48109

The circular mitochondrial genomes of the Oomycetes fall into two distinct organizational patterns with each pattern represented in at least two orders. The Achlya pattern is characterized by the presence of large inverted repeats which permit the genome to exist as two orientational isomers (1,2). Genome size variation of organisms with the Achlya pattern is primarily due to the recruitment of non-repeated sequences into the repeat resulting in genome expansion and gene duplication. The inverted repeats, by restriction enzyme analysis, are more conserved than the non-repeated sequences. The Phytophthora pattern (3) is more typical of fungal mitochondrial genomes in that the large inverted repeat is conspicuously absent. Gene orders, based upon hybridization to genic regions of Saccharomyces cerevisiae, will also be discussed.

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1609 FEATURES OF THE NEUROSPORA GENOME, David D. Perkins, Department of Biological Sciences, Stanford University, Stanford, CA 94305

Chromosome complements and meiotic chromosome behavior are strikingly similar in all Neurospora species, whether heterothallic, pseudohomothallic, or homothallic. As in other Sordariaceae the haploid number is 7, there is a single two-allele mating-type locus, and a single terminal nucleolus organizer. The synaptonemal complex is well defined, with recombination nodules. Chromosomes are large enough during ascus development to permit conventional analysis by light microscopy. Over 500 gene loci and 200 chromosome rearrangements have been mapped genetically in N. crassa, enabling each linkage group to be assigned to a visibly distinguishable chromosome. Chromosome rearrangements are abundant after mild mutagenesis in the laboratory. Chromosome ends are preferentially involved in rearrangements. Whole-arm translocations also occur. (Reviewed in refs 1-4.) Attention has been focussed on: a. Duplications (partial diploids) which are produced meiotically by insertional and terminal rearrangements (2). b. Vegetative (heterokaryon) incompatibility genes at numerous loci (5,6). c. Mendelian chromosomal elements called Spore killers. Ascospores receiving a Spore-killer-sensitive allele disintegrate postmeiotically in crosses of killer X sensitive (7,8). d. Rearrangements that involve the nucleolus organizer chromosome or the NO itself, so that dosage of rRNA genes can be altered (9,10). In one translocation having a divided NO, retranslocation occurs spontaneously as the result of legitimate meiotic recombination between blocks of rRNA genes in the displaced terminal and interstitial positions.

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1610 POSITION DEPENDENT AND INDEPENDENT REGULATION OF A GENE FROM THE SpoC1 GENE CLUSTER OF ASPERGILLUS NIDULANS. William E. Timberlake, Bruce L. Miller, Karen Y. Miller and Kellee Roberti, Department of Plant Pathology, University of California, Davis, CA 95616

We have previously described the structure and regulated expression of the SpoC1 gene cluster from Aspergillus nidulans (1). This 53 Kb segment of chromosomal DNA codes for at least 19 poly(A)$^+$ RNAs, some of which are transcribed from overlapping regions. The area of developmental regulation is approximately 37 Kb in length and is delimited by 1.1 Kb direct repeats. With one exception, RNAs transcribed from the central part of the cluster appear late during conidiophore development and accumulate specifically in spores. The exceptional transcript appears earlier during development and accumulates specifically in cells of the conidiophore. In contrast, RNAs encoded at the borders of the cluster occur in both somatic cells and spores. The results indicated that if a chromatin-level control mechanism operates to regulate expression of the SpoC1 gene cluster, as previously suggested by us (2), additional levels of regulation must also exist.

We have now examined the effect of repositioning one SpoC1 gene, designated C1-C, in the genome. A recipient strain of Aspergillus was constructed containing a null allele of the C1-C gene. A plasmid containing a wild-type copy of the gene and its 5' and 3' flanking regions was then introduced by transformation. Several transformants were selected in which the plasmid had integrated at sites other than the SpoC1 region. Blot analysis of RNA from these transformants indicated that the gene was expressed in somatic cells at a level much greater than when in the normal position. The repositioned genes nevertheless showed a considerable degree of developmental regulation. The results indicate that regulation of genes in the SpoC1 gene cluster involves both position dependent and position independent mechanisms.

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Cytoskeleton; Evolution; Industrial Fungi

1611 THE MOLECULAR CLONING AND EXPRESSION OF CELLULASE GENES FROM TRICHODERMA REESEI IN YEAST AND FUNGI, J.K.C. Knowles, P. Lehtovaara, I. Salovuori, T.T. Teeri and M. Penttilä

Trichoderma reesei secretes large amounts of different cellulolytic enzymes into the growth medium. A number of genes specifically induced when the fungus is grown on cellulose have been isolated in the vector λ1059 by their differential hybridisation to cDNA probes prepared from induced and repressed messenger RNA. Genes coding for Cellobiohydrolase I, II and Endoglucanase II and III have been identified by hybrid selection of messenger RNA and subsequently by DNA sequence analysis. Full length cDNAs coding for three of these enzymes have been isolated and are being transferred to the PGK yeast expression vector.

1612 DUAL AMATOXIN BINDING SITES IN THE WHEAT GERM RNA POLYMERASE II, Paul W. Morris and E. Michael August, Univ. of Ill. at Chicago, IL 60612.
The amatoxins are a family of bicyclic octapeptides synthesized by a host of basidiomycetes and accumulated to high levels by some members of Amanita and Galerina. The mechanism of toxicity involves non-covalent binding with class II RNA polymerase and subsequent inhibition of mRNA production. Chemical modification of amanitin at the tryptophan by diazotization or amination provides a series of derivatives retaining effective inhibition of the polymerase. Using these derivatives, we have examined the binding reaction with polymerase. Titration of the intrinsic fluorescense of the wheat germ polymerase II shows two binding sites. One site with $K_D = 10^{-7}$ M compares with the inhibitory site with $K_I = 10^{-7}$ M. The second site shows $K_D = 10^{-5}$ M. Each site has a stoichiometry of one. This finding of two binding sites on the wheat germ polymerase raises a question how the amatoxin-resistant polymerases of basidiomycetes, Drosophila, and animals differ from the sensitive polymerases. These amatoxin-resistant polymerases are characterized by a $K_I = 10^{-3}$ to 10^{-4} M. These values are suggestive of the low affinity binding site observed in the plant polymerase and question which parts of the protein sequence form the amatoxin binding site in each case. Do class II RNA polymerases generally contain two amatoxin binding sites and evolve to resistance by loss of the high affinity site?

1613 RANDOM FRAGMENT HYBRIDIZATION: A TOOL FOR STUDYING PHYLOGENETIC RELATIONSHIPS IN THE GENUS NEUROSPORA, Donald O. Natvig, Department of Biology, University of New Mexico, Albuquerque, NM 87131

Restriction-endonuclease analysis of DNA is rapidly becoming popular as a tool for studying phylogenetics. Analysis of mitochondrial DNA and repetitive nuclear DNA is readily accomplished, because restriction fragments can be resolved by gel electrophoresis. The same type of analysis is ordinarily not possible with single-copy regions of the nuclear genome, because the total number of fragments is too large to permit resolution of discrete sequences. However, restriction analysis of single-copy nuclear sequences is possible when it is combined with Southern hybridization. My recent research has employed cloned, single-copy sequences from Neurospora crassa (74A) as hybridization probes to detect polymorphisms among isolates of Neurospora representing four species. The results demonstrate that restriction analysis of random fragments (5-10 kbp) can provide a measure of genetic relatedness at a level of resolution far superior to isozyme electrophoresis or DNA:DNA solution hybridization. Experiments to date have employed heterothallic (8-spored) strains from three species and secondarily-homothallic (4-spored) strains assigned to N. tetrasperma. Strains of N. tetrasperma examined have been from Hawaii, Louisiana, Florida, Liberia and Bali. Relative to 8-spored strains, 4-spored strains collected worldwide are shown to be very closely related, suggesting that secondarily-homothallic strains are monophyletic. The analysis of 8-spored strains to date is insufficient to construct a non-ambiguous tree.

1614 A SPLIT GENE OF THE NEUROSPORA MITOCHONDRIAL GENOME IS HOMOLOGOUS TO THE MAMMALIAN URF5 GENE, Mary Anne Nelson and Giuseppe Macino, University of Rome, 00185 Rome, Italy

The human mitochondrial genome encodes about seven as yet unknown reading frames (URFs). We have determined the sequence of a gene homologous to the human URF5 gene in the mitochondrial genome (65 kbp) of Neurospora crassa, and the expression of this gene in wild type and mutant strains has been examined. The N. crassa URF5 gene is interrupted by two long intervening sequences; both introns contain extended open reading frames that are continuous and in frame with the respective upstream exon sequences. Transcript analysis has demonstrated that the URF5 gene is transcribed and that the large precursor transcripts are processed to yield an abundant potentially mature species of 3.2 kb. The 5' and 3' ends of the putative mRNA have been determined by S1 mapping and primer extension experiments, and those results will be presented. Mutant strains lacking all or part of the URF5 sequence have been examined. The characteristics of these mutant strains will be described, as will the results of a screening for point mutations in this and other URF genes in the Neurospora mitochondrial genome. The conservation of the URF sequences in the highly compact human mitochondrial genome suggests that the URF-encoded proteins serve important functions. Homologous proteins are encoded in the mitochondrial genomes of some fungi. The genetic approach that is possible in fungi may eventually allow us to determine the functions of these proteins.

1615 GENETIC STUDIES ON CEPHALOSPORIN C PRODUCTION BY ACREMONIUM CHRYSOGENUM John F. Peberdy, Univ Nottingham, University Park, Nottingham NG7 2RD England

A protoplast fusion cross between divergent lineages of raised titre strains of Acremonium chrysogenum produced a number of haploid recombinants and heterozygotic colonies. Spontaneous haploidization of the latter also gave progeny that were analysed. Recombination of auxotrophic markers was observed. All the progeny were assessed for penicillin N and cephalosporin C production. The population of haploid recombinants derived from the fusion plates gave a broader distribution of titres than did the haploids recovered from heterozygotes, suggesting possible clonal effect in the latter. Many of the recombinants displayed a greater efficiency in the conversion of penicillin N to cephalosporin C than the parental strains. Linkage of "effective factors" to auxotrophic markers is discussed.

1616 CHARACTERIZATION OF THE 582 REPETITIVE ELEMENT IN THE *DICTYOSTELIUM DISCOIDEUM* GENOME. Hans Richter and Herbert L. Ennis, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110

Approximately 70-75% of the *Dictyostelium discoideum* genome is single copy and the remaining 25-30% is composed of sequences that are repeated 50 to several hundred times per haploid complement. Although the role of these repetitive elements is unknown, recent evidence indicates that they may be transposons and may be involved in the regulation of transcription in *Dictyostelium*. A repetitive DNA sequence was isolated from a *Dictyostelium* genomic plasmid library of Bgl II digested DNA ligated to the Bam HI site in pBR322. This clone, called 582, hybridizes to a large number of phage λ *Dictyostelium* genomic clones which have been characterized. Southern blot analysis shows that 582 hybridizes to many differently sized genomic DNA fragments generated by digestion with Eco RI and Hind III. In addition, restriction mapping of 582 and six genomic clones shows that the flanking regions of each of the genomic clones are different. These findings indicate that the sequences specific to 582 are scattered throughout the *Dictyostelium* genome. Northern blot analysis reveals that RNA (3 kb) which hybridizes to 582 DNA is present during all stages of growth and development and does not seem to be developentally regulated. Southern blot analysis of DNAs from *D. giganteum, D. purpureum* and *Polyphondylium violaceum* were performed to determine whether the 582 sequence is present in other species of slime molds. Hybridization of 582 was observed to DNA from all the *Dictyotelium* species but not to *Polysphondylidium*. This approach might be used as a biochemical parameter for the molecular taxonomy of the cellular slime molds.

1617 EXTENSIVE METHYLATION AND NUMEROUS TRANSITION MUTATIONS IN TANDEM PAIR OF NEUROSPORA 5S RNA GENES, Eric U. Selker, Judith N. Stevens and Robert L. Metzenberg, University of Oregon, Eugene, Oregon 97403 and University of Wisconsin, Madison, Wisconsin 53706.

Neurospora crassa has at least six types of 5S RNA genes each with very distinctive 5S coding regions. Although not entirely randomly distributed, they are generally widely dispersed, and are on at least six of the seven chromosomes of Neurospora (see Metzenberg et al., this volume). However, one 5S gene clone (of 22 analyzed in detail) contains two 5S genes (ζ and η) separated by about 700 nucleotides. DNA sequence analysis suggests that ζ and η arose by a duplication followed by the accumulation of mutations at about 15% of the positions in both the 5S coding and flanking DNA. Remarkably, all of the changes can be accounted for by C to T mutations in one strand or the other. A simple explanation is that the mutations resulted by deamination of methylated cytidines. An implication is that at one time or another, many and possibly all of the C residues in this region were methylated. We find that this region (but not seven other Neurospora 5S genes examined) is still extensively methylated in our standard lab strain. Another strain has only one 5S gene in this region, and it is not methylated. From a cross of these strains, all progeny having both ζ and η also have the DNA methylated in this region. Thus a nucleotide sequence difference between the strains in the ζ-η region segregates with the methylation difference. We suggest that DNA methylation may be a response to tandem duplication in Neurospora.

1618 MITOCHONDRIAL DNA AND EVOLUTION OF THE GENUS NEUROSPORA, J.W. Taylor, G. May and B.D. Smolich, Department of Botany, University of California, Berkeley, CA 94720

We are studying mitochondrial DNA variability in the five heterothallic Neurospora spp. to understand their phylogenetic relationships. Our initial experiments involve 20 isolates of N. crassa, 14 from 3 contiguous sites in Louisiana and 6 from other parts of the world. Restriction endonuclease site maps for each isolate, made by hybridization between the mtDNAs of natural isolates and N. crassa 74A mtDNA, show large variability within the species, some due to nucleotide substitution but most due to length mutations (insertions and deletions). Our maps are conservative as the larger length mutations may be composed of several smaller length mutations (Collins and Lambowitz 1983, Plasmid 9:53-70). mtDNAs of the 20 isolates can be accommodated by 7 length mutation types. The most common mtDNA type, represented by 10 isolates drawn from the three Louisiana sites, differs from N. crassa 74A by a 1.0 kbp deletion in EcoR I fragment 5 and an 80 bp insertion in EcoR I fragment 9. mtDNAs of the other Louisiana isolates exhibit only one or the other of these two length mutations. All of the other isolates, except one isolate from Costa Rica, have additional length mutations; isolates from Florida and the Ivory Coast shown an extreme of 7 mutations. These mutations appear to be correlated with geographic distribution, implying that intraspecific evolution can be studied by mtDNA analysis. The low amount of intraspecific nucleotide substitution predicts that studies of species, genera and even higher taxa will be worthwhile. mtDNA variability is probably not large enough to make studies of individual isolates possible as identical mtDNA have been found between and within populations in Louisiana. Preliminary data on comparison of the heterothallic species will also be presented.

Molecular Genetics of Industrial Fungi

1619 MOLDS, MANUFACTURING, AND MOLECULAR GENETICS: PERSPECTIVES AND PROMISES. J. W. Bennett, Department of Biology, Tulane Univerisity, New Orleans, LA 70118.

Industrial microbiology is a well established factor in the world's economy, valued in the tens of billions of dollars. Industrial processes involving microorganisms include the production of: microbial biomass and enzymes; low molecular weight primary and secondary metabolites; nonmicrobial proteins by genetically engineered organisms; and finally, the biotransformation of selected chemicals. The best known fermentation processes involve yeasts, which have been used in brewing and baking for millenia. Filamentous fungi have also found ancient applications, particularly in the Orient, where molds are involved in the production of soy sauce, sake, tempeh, and other traditional foods and beverages. The role of filamentous fungi in the development of modern fermentation industries is less well known. Many of the original patents for microbial processes involve species of Aspergillus and Mucor, i. e., the first U. S. patent for a fungal, commercial enzyme was issued in 1894 for "Takadiastase" from A. flavus-oryzae. The feasibility of commercial citric acid production from A. niger was demonstrated in 1917. However, it was only after World War II, with perfection of commercial fermentations for the manufacture of penicillin, that we entered the modern era of industrial microbiology. Development of the penicillin process led to an understanding of how to achieve large scale sterilization of media and equipment; how to provide proper aeration and culture conditions; how to preserve inocula; and, not least, how to isolate improved strains. Virtually all strain improvement to date has been effected by a simple strategy of brute force mutagenesis, screening, and selection. This empiric process is slow, painstaking, expensive, and successful. Its success has hampered more rational approaches.

Molecular genetics offers new promises. Filamentous fungi will serve two major roles in modern biotechnology. In one role, they will be the source of unique fungal products. Fungal genes can be isolated, cloned, and expressed in bacteria and yeasts. This is state-of-the-art technology for a number of fungal amylases, cellulases, and proteases. In their second role, molds will be hosts for engineered fermentation processes. This will allow us to exploit the scale-up technologies developed for citric acid and penicillin fermentations. More excitingly, it will allow us to engineer multi-step biochemical pathways in situ. Directed manipulations of fungal genetic material will be sought to increase yields of existing, commercially important primary and secondary metabolites, as well as to offer the promise of novel, pharmacologicly active drugs from filamentous fungi.

1620 THE GENETIC MANIPULATION OF FUNGI THAT PRODUCE ANTIBIOTICS, John F. Makins, Genetic Microbiology, Bristol-Myers Co., P.O. Box 4755, Syracuse, N.Y. 13221-4755.

Penicillium chrysogenum and Acremonium chrysogenum are both industrial microorganisms used in the commercial production of the β-Lactam antibiotics penicillin and cephalosporin. While these species have been the subject of intensive genetic modification in strain improvement programs[1] relatively little is known about their genetic constitution at levels of either classical or molecular genetics.

In contrast, Aspergillus nidulans which also produces penicillin, (albeit in small amounts) has been a model system for classical genetic studies for 30 years and is consequently a well understood microorganism.[2]

For example, probably all the steps involved in the biosynthesis of penicillin, are defined in mutant lines of A. nidulans available from various culture collections. With the advent of plasmid cloning vectors for A. nidulans[3][4][5] the genes governing these steps can be isolated by selecting or screening for transformants which have been "rescued" from the mutant phenotype. As parallel techniques are developed to enable the stable introduction and expression of recombinant DNA molecules into C. acremonium[6] and P. chrysogenum then it will be possible to directly manipulate the biosynthesis of β-lactam antibiotics by fungi.

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1621 PROTOPLAST FUSION AND HYBRIDIZATION IN PENICILLIUM, F.M. Mellon*, J.F. Peberdy** and K.D. Macdonald***. *School of Biological Sciences, Queen Mary College, Mile End Road, London, E1 4NS. **Department of Botany, University of Nottingham, University Park, Nottingham, NG7 2RD. ***Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, SP4 0JQ.

Fungal protoplasts have proved to be very useful in genetic studies over the last decade. The initial interest was in interspecific protoplast fusion, particularly in the Aspergilli and Penicillia. Fusion of protoplast was found to be induced by polyethylene glycol (PEG) and fusion products were first isolated following nutritional complementation of auxotrophic markers. More recently selection techniques involving the use of dead donors and drug resistance have been developed. Fusion products can be characterized genetically by haploidization analysis and biochemically by analysis of the mitochondrial genomes, isoenzyme banding patterns and antibiotic production.

In recent years protoplasts have formed the basis of fungal transformation systems in a variety of species. An important prerequisite of these systems is the need to have a reproducible protoplasting procedure in terms of the numbers of protoplasts isolated and their regenerative capacity.

1622 DEVELOPMENT OF THE GENETICS OF THE DIMORPHIC YEAST YARROWIA LIPOLYTICA, David M. Ogrydziak and Rod A. Wing, Institute of Marine Resources, University of California, Davis, CA 95616

Sexuality of Y. lipolytica (previously Candida, Endomycopsis or Saccharomycopsis lipolytica) was first reported in 1969 by Wickerham, Kurtzman, and Herman (1). They established that Y. lipolytica was heterothallic and they published procedures for mating, sporulation, and stabilization of haploids and diploids. Inbreeding programs in R. Mortimer's and H. Heslot's laboratories resulted in improvements in the percentage of four-spored asci and in spore viability which made tetrad analysis feasible (2, 3). Most of the classical genetic techniques which have been developed for Saccharomyces cerevisiae are now possible with Y. lipolytica. A primitive genetic map consisting of five linkage fragments on which a total of twenty six genes have been mapped has also been developed (4).

At least two integrative transformation systems have been developed for Y. lipolytica. One system used the Y. lipolytica LEU2 gene inserted into pBR322 for the vector and a Y. lipolytica leu2 host. The lithium acetate transformation procedure was used and transformation frequencies as high as 100 transformants per microgram of uncut DNA and 10^4 per microgram of DNA linearized with restriction enzymes were obtained. The other system used Y. lipolytica DNA inserted into YIp333 (which carries the S. cerevisiae LYS2 gene) for the vector and a Y. lipolytica lys2 host. The spheroplast transformation procedure was used and transformation frequencies of 1-10 transformants per microgram of uncut DNA were obtained.

No Y. lipolytica autonomously replicating sequences have yet been isolated despite attempts in three different laboratories. We have inserted random fragments of Y. lipolytica DNA into the LEU2 (from Y. lipolytica)/pBR322 hybrid vector and transformed a leu2 host. Slow growing, unstable transformants were obtained. When Southerns of DNA isolated from these transformants were hybridized with pBR322 a broad band with the mobility of chromosomal DNA and a sharper band of greater mobility (possibly plasmid) were detected. However, attempts to recover the plasmids by transformation of E. coli have not yet been successful.

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Fungal Differentiation

1623 A WALL AT THE END OF THE LONG ROAD OF MOLECULAR MYCOLOGY, Salomon Bartnicki-Garcia, Department of Plant Pathology, University of California, Riverside, CA, 92521

This largely speculative presentation will analyze some basic concepts and tenets of fungal morphogenesis. Is all the information needed to make a fungus stored in DNA sequences? How does this information eventually find its way to the targets or sites responsible for the shape of a fungal cell? The current dialect of molecular genetics describes, in fascinating detail, many crucial events in the linear transfer of molecular information from DNA to RNA to protein. But does the resulting conglomerate of enzymes and structural proteins contain all the molecular information needed to make and shape a fungus cell? Specifically, can these proteins alone specify the three dimensional structure of the ultimate product of an individual fungal cell -- its cell wall? Most of what we know about the structure and construction of subcellular organelles is expressed in the dialect of cell biology. Our challenge is to find the keystone(s) that bridge(s) the gap between molecular and cell biology. The proposed role of vesicles (particularly chitosomes) in fungal growth and morphogenesis (1-3) will serve as a focal point of this discussion.

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1624 FUNGAL SPORE GERMINATION AND MITOCHONDRIAL BIOGENESIS, Robert Brambl, Department of Plant Pathology, The University of Minnesota; Saint Paul, Minnesota 55108

The germination of fungal spores appears to be dependent upon function of the mitochondrial respiratory membrane. Spores of two fungi we have examined meet this requirement differently. The conidia of Neurospora crassa contain a complete and apparently functional respiratory membrane that is active at the onset of spore germination [1,2]. Despite the presence of a preserved respiratory membrane, however, these spores also contain a population of preserved mRNA with a transcript for a representative subunit, the proteolipid subunit of the ATPase. This population of preserved poly A(+)RNA can be translated in vitro into the subunit peptides of cytochrome oxidase and ATPase.

The dormant spores of Botryodiplodia theobromae contain mitochondria that do not have a functional respiratory membrane; most of the cytochrome components are absent, as are catalytic activities of cytochrome oxidase and ATPase [3,4]. Study of two enzymes has demonstrated two mechanisms by which the mitochondrial respiratory membrane of Botryodiplodia spores is reorganized upon initiation of spore germination. The subunits of cytochrome oxidase are preserved during dormancy in the separate cellular compartments of synthesis. Upon initiation of germination the cytoplasmically synthesized subunits are rapidly mobilized from the cytoplasm for translocation into the mitochondria where they assemble with mitochondrially synthesized subunits and heme a to yield a functional enzyme [4]. A reversible inactivation of the mitochondrial membrane system responsible for translocation of these cytochrome oxidase subunits into the mitochondria may be a sporulation-induced cellular rearrangement that leads to exclusion of these subunits and their accumulation in the cytoplasm during dormancy. Mitochondria isolated from dormant spores cannot incorporate cytoplasmic subunits of cytochrome oxidase, but this defect is corrected early in germination. In contrast, none of the subunits of the mitochondrial ATPase are preserved in the dormant spores. Instead, the subunits of this enzyme are translated de novo from messenger RNA preserved in the dormant spores to yield an active enzyme immediately after initiation of germination [2]. The poly A(+)RNA fraction of these spores can be translated in vitro into peptides that are precipitated with antiserum to the enzyme.

Studies of the assembly of subunits of cytochrome oxidase and ATPase in germinating spores of Neurospora have revealed an unusual type of post-translational modification of certain subunits of cytochrome oxidase and ATPase with a derivative of pantothenic acid [5]. In the absence of pantothenate, the catalytic activities of the two enzymes do not increase above the initial activities, and although all subunits of these enzymes accumulate in the mitochondria, they fail to assemble into normal enzyme complexes.

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1625 GENETIC CONTROL OF SPORULATION IN ASPERGILLUS NIDULANS. Sewell P. Champe, Waksman Institute of Microbiology, Rutgers University, New Brunswick, NJ 08903. The homothallic ascomycete Aspergillus nidulans produces two kinds of spores at well-defined and distinctly different stages of the life cycle. For spore-initiated cultures growing at 37 C, asexual conidiospores (conidia) are produced early, beginning at 20 h, and rapidly increase in number until 40 h. Sexual fruiting bodies (cleistothecia) begin to appear shortly after conidiation ceases and begin to bear sexual ascospores at about 80 h. Mutational analysis has identified three genes, acoA, acoB and acoC which are required at a very early stage of conidiation and which are required also for later sexual development (1,2). Mutants in each of these three genes display the same metabolic defect which is manifested by the accumulation of a set of 15 or more phenolic metabolites at levels much higher than the wild type or mutants blocked at later stages of conidiation. If purified individual metabolites are applied to wells in confluent lawns of wild-type A. nidulans, several biological effects are observed, depending on the metabolite: 1) inhibition of spore germination and hyphal growth, 2) inhibition of conidiation but normal hyphal growth, and 3) stimulation of premature sexual development as evidenced by the early appearance of cleistothecia. The growth inhibitor has been identified as 3,3'- dihydroxy-5,5'-dimethyldiphenyl ether, and analysis of the other bioactive compounds is in progress. We are exploring the possibility that the compounds accumulated by the mutants may play a role in the normal induction and regulation of sporulation.

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1626 FUNGAL DIFFERENTIATION: PROBLEMS AND PROSPECTS, James S. Lovett, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

The introduction to the session on Fungal Differentiation will summerize the background for present experimental approaches to fungal differentiation and development and emphasize major problems that still require resolution.

There is a very large body of information on the environmental and nutritional conditions that can induce asexual and sexual development of various fungi. This abundant work has demonstrated that the carbon and nitrogen sources available may influence the shift from growth to a developmental phase. It has not yet led to useful generalizations on the mechanisms of induction, nor provided much insight on how nutritional factors and other developmental stimuli may effect the same response. Considerable effort has also been expended on studies of intermediary metabolism during fungal development. These changes, though normal accompanyments of differentiation, are probably secondary events and may be largely due to changes in the nutritional status that are associated with developmental induction. Genetic approaches have been fruitful in the identification of genes for some enzymes associated with development, and for mutants blocked at various stages (e.g., conidiophore and conidium development), as well as in earlier events leading to sporulation competence. Such work, particularly with conditional mutants, should continue to produce useful information on both the details of structure formation and toward the identification of regulatory systems. The results of molecular hybridization with stage-specific poly(A)RNA preparations and cDNAs have indicated that a significant fraction of the fungal genome may be differentially expressed during development, but cannot provide information on the specific genes or their products.

Although progress has been made, many important questions remain unanswered concerning almost all aspects of fungal development. Experimental work directed toward answers to some of these questions will be discussed in the papers to follow, but it seems evident that we are still a long way from a complete characterization of the structural and synthetic details, or the underlying regulatory mechanisms, for any single developmental structure in any of the "simple" eukaryotic systems presented by the fungi.

1627 GENE EXPRESSION DURING BASIDIOCARP FORMATION, Joseph G.H. Wessels, Department of Plant Physiology, Biological Center, University of Groningen, Haren, The Netherlands.
Mating of two haploid monokaryons of *Schizophyllum commune* with different alleles at the *A* and *B* incompatibility genes results in a stable dikaryon that readily produces basidiocarps, in contrast to the monokaryons. A mutational analysis of these incompatibility genes has revealed that they act as master switches controlling genes operative in the monokaryon-dikaryon transition (1). At the molecular level, however, few differences can be detected between vegetative monokaryotic and dikaryotic mycelia. The patterns of protein synthesis differ slightly but RNA/scDNA and RNA/cDNA hybridizations as well as *in vitro* RNA translations failed to indicate differences in total or polysomal RNA populations, with the exception of a possible difference in polyadenylation of certain sequences (ref. cit. in 4). In contrast, conspicuous differences in the synthesis of some abundant proteins arose when the dikaryon proceeded to form basidiocarps. Proteins not seen in the monokaryon were synthesized in the dikaryon, in basidiocarps, in both basidiocarps and supporting mycelium or excreted into the medium (2). At the same time, RNA/cDNA hybridization and *in vitro* RNA translation showed the appearance of 25-35 novel abundant mRNAs in the dikaryon but regulation in the class of rare mRNAs could not be detected (3). A number of the fruiting-specific mRNAs were cloned. One of these cDNA clones, corresponding to a very abundant mRNA of 650 nt, and its genomic clone, were studied in some detail (4,5) revealing the coding sequence and three very small introns (53, 49 and 49 nt) in the gene. The use of a number of fruiting-specific cDNA clones to probe the concentration of the corresponding mRNAs revealed a sharp increase at the time of fruiting, some mRNAs accumulating in the basidiocarps, others in the supporting vegetative mycelium or in both. None of these fruiting-specific mRNAs was found in the parent monokaryons but low concentrations could be detected in the derived vegetative dikaryon. It thus appears that the presence of two different alleles of the incompatibility genes in the dikaryon conditions fruiting genes to become expressed but full expression only occurs when basidiocarps are actually formed.

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Fungal Differentiation; Fungal Pathogenicity to Plants

1628 MULTIPLE ELICITORS FROM THE FUNGAL PATHOGEN, COLLETOTRICHUM LINDEMUTHIANUM
A. J. Anderson and C. S. Tepper, Utah State University, Logan, Utah 84322

Colletotrichum lindemuthianum exists as a group of races with differential virulence on selected bean cultivars. Resistance in non-hosts frequently involves changes in the challenged plant cell termed a hypersensitive response. Changes characteristic of hypersensitivity are incited in plant tissue treated with fungal components called elicitors. Glucans from isolated hyphal walls of three races of C. lindemuthianum were elicitors at concentrations of 1 μg glucose equivalents/ml on several bean cultivars. These glucans cannot explain race-cultivar specificity although they may function in resistance in plants other than bean. In contrast, extracellular components from α and β races were elicitors only on resistant and not susceptible cultivars. Purification of β race products failed to generate fractions with elicitor activity on resistant cultivars or an ability to suppress elicitor activity from wall glucans or α race extracellular products. Chromatography of α race products revealed elicitor-active fractions E_1-E_3. E_1 was not adsorbed to DEAE- or CM-Sephadex and had a high carbohydrate to protein ratio. E_2 and E_3 eluted as distinct peaks from DEAE-Sephadex and had higher protein to carbohydrate ratios. Other KCl-eluted fractions lacked elicitor activity. Fractions E_2 and E_3 showed a unique band at 90,000 D on SDS-PAGE after silver staining. E_1-E_3 were active at 50-100 pg protein/ml only on resistant cultivars. Sugar compositions of E_1 E_2 and E_3 were similar yet distinct from α and β race non-elicitor products. Antibodies against E_1 E_2 and E_3 are being used to further probe molecular and genetic differences between α and β races.

1629 EXPRESSION AND CHROMOSOMAL DISTRIBUTION OF GENES INDUCED DURING CONIDIATION IN NEUROSPORA CRASSA, Vivian Berlin and Charles Yanofsky, Stanford University, Stanford, CA 94305

A Neurospora genomic DNA library was screened with a cDNA probe enriched in sequences expressed in conidiating cultures. Clones were isolated that preferentially hybridized to this probe versus a second cDNA probe complementary to poly (A)$^+$ RNA isolated from mycelia. Twelve clones contained unique sequences that hybridized to 22 transcripts; of these, 19 accumulated preferentially in conidiating cultures. Eight transcripts were present at higher levels in conidiating cultures than mycelia. Eleven were present only in conidiating cultures and were first detected at different times during the asexual cycle. We mapped genomic sequences homologous to the eleven clones by conventional crosses, using restriction fragment length polymorphisms as genetic markers. The sequences homologous to genes expressed preferentially in conidiating cultures are distributed on six of the seven chromosomes. Clones that map to the same chromosome are linked. No recombination occurred between genomic sequences homologous to three clones suggesting the genes contained in these clones may constitute a gene cluster.

1630 MOLECULAR GENETICS OF PATHOGENICITY IN COCHLIOBOLUS CARBONUM, Steven P. Briggs, Richard W. Bass, Susan L. Jones, Pioneer Hi-Bred International, Inc., Johnston, IA

We have defined a locus, Tox1, which controls production of host-selective toxin by C. carbonum. A recombination analysis with other races and species (C. victoriae) which fail to produce the toxin revealed that all non-producers are defective at only the Tox1 locus. This locus exhibits a high mutation frequency. A genetic transformation system is being developed to clone Tox1. Three different C. carbonum arg mutants have been treated with pBR322, Yep24, and pYe(ARG4)411. One of the mutants generates colonies on minimal medium but only if treated with pYe(ARG4)411 (contains the yeast gene for argininosuccinate lyase). Growth on complete medium causes reversion to auxotrophy. Tests for incorporation of foreign sequences are under way. Attempts to clone the gene corresponding to ARG4 from Cochliobolus have been partially successful. E. coli argH (= arg4) has been transformed to prototrophy using Yep24 libraries of C. heterostrophus provided by O.C. Yoder. However, the marker rapidly recombines into the chromosome or else (in recA strains) is very unstable. One prototrophic cell in liquid minimal medium can feed 1000 auxotrophic cells, so attempts to prepare homogeneous plasmid have failed due to excision of the arg gene from most of the plasmid population.

1631 FUNGAL POLYGALACTURONASE AS A FACTOR OF SPECIFICITY IN PLANT DISEASE, F. Cervone and G. De Lorenzo, Plant Biology Dpt., University "La Sapienza", Rome - Italy

Specific binding of polygalacturonase (PG) is a factor of recognition between a plant tissue and a fungus during the first stages of infection. French bean tissue binds PG from Colletotrichum lindemuthianum and does not bind PG from fungi non pathogenic to bean. Races of C. lindemuthianum exhibit a kind of specificity towards bean cultivars which so far has not found a molecular explanation. In order to investigate whether PG mediates the specific interaction between plants and fungi,the enzyme was purified to homogeneity from α and β races of C. lindemuthianum. The purified enzymes had similar enzymological and structural properties. Nevertheless,they showed a differential rate of absorption to the tissues from 4 bean cultivars.The rate of absorption was faster in the resistant combinations than in the susceptible ones.As the resistant response is considered a rapid and localized susceptible response, a fast absorption of PG to the tissue is consistent with a process of rapid cell death,necrosis of the tissue surrounding the infection site and a consequent hypersensitive response. It is worth mentioning in this connection that PG's act in some cases as elicitors of phytoalexin synthesis.A differential rate of PG absorption may determine the rapidity of phytoalexin accumulation and consequently whether a combination is resistant or susceptible.

1632 TRANSFORMATION AND GENE CLONING IN MAGNAPORTHE GRISEA. F.G. Chumley, K.A. Parsons, and B. Valent. Department of Chemistry University of Colorado Boulder, CO 80309. We have begun to develop a system for transformation and gene cloning in M. grisea (anamorph, Pyricularia oryzae or P. grisea), a fungal pathogen of many grasses. Our goal is to clone genes that govern pathogenicity and virulence in this organism, where individual isolates show restricted host range. These cloned genes will be used to analyze the molecular genetic basis for differences in the ability to attack various species of grasses or various cultivars of rice. M. grisea is a filamentous Ascomycete that grows on defined media. We have isolated many auxotrophic mutants; among those that require arginine we have identified one with a defect in the gene encoding ornithine carbamoyl transferase (OCTase). Strains that carry this mutation will be used as recipients in transformation experiments with donor plasmids containing the A. nidulans argB OCTase gene. This gene is expressed in Neurospora and yeast, and it will probably function in M. grisea as well. Other transformation attempts will focus on M. grisea gdh mutants, which lack NADP-dependent glutamate dehydrogenase activity (GDH). These experiments will involve donor plasmids with the Neurospora am GDH gene. M. grisea gene banks have been constructed, and we are using yeast, A. nidulans, and E. coli as intermediate hosts for the isolation of M. grisea genes that can be used as homologous donors in transformation of M. grisea recipients. Vectors and approaches for cloning of pathogenicity determinants will be discussed. (This work supported by the USDA and by the Monsanto Agricultural Products Company.)

1633 PARTIAL PURIFICATION OF PISATIN DEMETHYLASE FROM NECTRIA HAEMATOCOCCA, Anne E. Desjardins and Hans D. VanEtten, Cornell University, Ithaca, NY 14853

Some isolates of the fungus Nectria haematococca can demethylate pisatin, a phytoalexin from pea. Pisatin demethylation is necessary for tolerance to pisatin and virulence on pea, and is catalyzed by a microsomal cytochrome P-450 monooxygenase system. We have solubilized and reconstituted pisatin demethylase from microsomal membranes. We now report further purification and characterizaton of the components of this enzyme system. Using a combination of chromatography on Blue A-agarose, aminohexyl-agarose and ADP-agarose, we have purified NADPH-cytochrome c reductase to a specific activity of approximately 20 μmoles per min per mg, with a yield of 25%. A molecular weight of 84,000 was estimated by sodium dodecylsulfate polyacrylamide gel electrophoresis. The cytochrome P-450 that catalyzes pisatin demethylation has been partially purified by the same procedures. Upon gel electrophoresis, the preparation gave several minor bands and three major bands of molecular weights 58, 56 and 52,000. The cytochrome P-450 lost all demethlase activity during concentration after amino-hexyl chromatography, so could not be purified further. The purified reductase can reconstitute demethylase activity of cytochrome P-450 fractions, and appears to be rate-limiting for demethylase activity in microsomal extracts.

1634 POLYMORPHISM OF ACID PHOSPHATASE ISOZYMES IN ECTOMYCORRHIZAL FUNGI, Iwan Ho and J.M. Trappe, Forestry Sciences Lab., Pacific NW Forest & Range Experiment Station, Forest Service, U.S.D.A.

Mycorrhizal fungi are characterized by considerable variations between species and within species. This tendency reflects mechanisms that are controlled by adaptive genes and modified by the environment factors. Thirteen species and thirty nine isolates of Amanita, Rhizopogon, Suillus, Laccaria, and Pisolithus collected from widely separated geographical areas were compared for acid phosphatase isoenzymes. The acid phosphatase emzyme system active in mycorrhizal fungi appeared to be particularly related to ecologically different parts of the range of the host tree species. The edaphic ecotypic relavance of genetic polymorphism of acid phosphatase were apparent and different alleles were found to be dominant with different host tree species.

1635 **ANALYSIS OF VARIATION IN BREMIA LACTUCAE.** Scot H. Hulbert and Richard W. Michelmore, University of California, Davis, CA 95616.

Bremia lactucae is a diploid, Oomycete fungus which exhibits a clear gene-for-gene relationship with its host, Lactuca sativa (lettuce). In common with all plant pathogenic fungi very little is known about the mechanisms of variation. Large numbers of markers are being developed using restriction fragment length polymorphisms. Random fragments of low copy number, genomic sequences and heterologous cDNAs are being used as probes. The segregation of these markers will be analyzed in somatic variants. This will demonstrate whether changes in virulence phenotype result from somatic crossing over, chromosomal struotural changes or heterokaryosis. Also, the frequency of polymorphisms at marker loci linked and unlinked to virulence genes will be compared. This will indicate whether regions of the genome determining virulence are evolutionarily unstable and will provide clues to the generation of novel virulence in pathogen populations.

1636 RNA SPECIES COMMON TO CONIDIA AND ASCOSPORES OF A. NIDULANS, James E. Jurgenson, Rutgers University, Piscataway, New Jersey 08854

Aspergillus nidulans produces asexual conidia and sexual ascospores. From the studies of Timberlake it is known that certain mRNA species are packaged selectively into conidia. I have examined ascospores to determine whether the same species found in conidia are also present in ascospores. Using a set of lambda clones containing conidia-specific genes I have found that most such clones did not detectably hybridize with cDNA prepared from ascospore poly A+ RNA. However, a few hybridized weakly, and one clone, Spo28, hybridized to the same extent as to cDNA prepared from conidial RNA. Thus at least some of the mRNA species that are packaged in conidia are also packaged in ascospores. The aconidial mutants bristle and abacus produce viable ascospores in normal amounts, but these ascospores lack the Spo28 transcript. The presence of this transcript in wild-type ascospores thus does not appear to be necessary for the formation or germination of the spore.

1637 ROLE OF THE PHYTOTOXIN : TENUAZONIC ACID, IN THE RICE BLAST DISEASE. MH. LEBRUN, J. ORCIVAL, S. RANOMENJANAHARY, F. GAUDEMER, A. GAUDEMER, M. VALES°, Dept Cryptogamy and Chemistry, PARIS 11 University, 91405 ORSAY, FRANCE, °IRAT, BP 5035, 34032 MONTPELLIER, FRANCE.

Tenuazonic acid (TA) is a phytotoxin produced by an ascomycete : Pyricularia orizae (P.O.) responsible for a major disease of rice. This toxin has already been detected in phytotoxic amounts in infected rice leaves. Its toxicity is thought to arise from protein synthesis inhibition at the ribosome level, but whether this toxin is involved in the aggressiveness of the pathogen is not known. To study this aspect of the plant-pathogen interactions we developped three programs :

1/TA. Chemistry : we studied the physicochemical properties of TA (metal complexation) and relation between toxicity and structure with synthetic analogs. Chromatography has been used to separate TA from its natural analogs.

2/Genetics of P.O. We are screening TA non producers from international collections. Up to now, only one non-producer, from Japan, have been detected with moderate aggressivity. Isolation of TA non-producers, after mutagenesis, to evaluate the role of the toxin in pathogenesis is underway.

3/TA mode of action. TA is a non-selective toxin since it is toxic at the same concentration for all rice genotypes. Some varieties resistant to P.O. react to TA applied on leaves by a local brown pigmentation around the point of application. There is a good correlation between the level of partial resistance to P.O. and the speed of the browning effect on leaves.

1638 GENETICS AND CYTOLOGY OF THE RICE BLAST FUNGUS, PYRICULARIA ORYZAE. Hei Leung and Paul H. Williams, University of Wisconsin, Madison, Wisconsin 53076.

Pyricularia oryzae (Ascomycete) parasitizes a variety of gramineous hosts and causes rice blast disease. Although a high degree of pathogenic variability has been reported in the fungus, little is known about the nature of this variability. Genetic and cytological studies were therefore made as a prerequesite to molecular studies on pathogenic variation. Through matings among isolates from rice, finger millet, and weeping lovegrass, the inheritance of electrophoretic variants of six enzymes, phosphoglucomutase (Pgm), phosphoglucose isomerase (Pgi), glycerate-2-dehydrogenase (G2dh), malate dehydrogenase-3 (Mdh3), lactate dehydrogenase-3 (Ldh1 and Ldh3) was determined. All six variants were under single gene control as determined by tetrad and random spore analysis. However at Ldh3 and Mdh3, there were consistent excesses of variant alleles among ascospore segregants. Genetic analysis of hermaphroditism suggested that maleness in two Japanese rice isolates might be due to single gene mutation. Linkage analyses among the six electrophoretic markers, mating type and hermaphroditism suggested loose linkage between Pgm and G2dh with a recombination frequency of 43.0%. Ascospore progeny derived from rice isolates were either non-pathogenic or only partially pathogenic on rice but retained pathogenicity to weeping lovegrass. Meiosis and mitosis in P. oryzae resembled that in other ascomycetes. Six chromosomes were observed at pachytene, diakinesis and metaphase of ascospore mitosis. The longest chromosome at pachytene averaged 8.5 um and the smallest chromosome averaged 2.9 um. Chromosome movement at meiotic and mitotic anaphase was asynchronous which might account for the variable chromosome numbers reported previously.

1639 REGULATORY PHENOTYPES OF PISATIN DEMETHYLASE IN NECTRIA HAEMATOCOCCA IN REGARD TO PATHOGENICITY ON PEA AND TOLERANCE TO PISATIN. Susan Mackintosh and Hans VanEtten, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853-0331.

Previous genetic studies have shown that the ability of N. haematococca to demethylate the pea phytoalexin pisatin is required for a high level of tolerance to this phytoalexin and for virulence on pea (Pisum sativum). In addition, studies of demethylating (pda^+) fungal isolates with large differences in inducible enzyme levels suggested that the relative rate of pisatin demethylation is associated with differences in tolerance and virulence (J. Gen. Microbiol. 130:2595). To examine this relationship further, genetic analysis was done on isolates with small differences in inducible enzyme activity. These differences were measured by following the time course of pisatin demethylation over a 48 h period. Isolates were identified which demethylated all of the pisatin (0.1 mM) in 6 to 12 hours, while other isolates required > 30 h. A cross between isolates with these two pda^+ phenotypes produced pda^- progeny in addition to progeny with the two parental phenotypes, suggesting that different genes are responsible for the pda^+ phenotypes. Preliminary studies have failed to detect a significant difference in pisatin tolerance between the two pda^+ phenotypes. However, all highly virulent progeny have the more rapidly demethylating phenotype.

1640 MORPHOGENESIS AND VIRULENCE IN HISTOPLASMA CAPSULATUM, Gerald Medoff, Judith Medoff and Alan Lambowitz, Washington University and St. Louis University, St. Louis, Missouri 63110

H. capsulatum is a dimorphic fungal pathogen that grows as a multicellular mycelium in soil and as intracellular yeast in infected humans and animals. Morphogenesis and the factors that affect it are of considerable interest because growth of the yeast phase appears to be required for pathogenicity. We have studied the transition from mycelial to yeast morphology in one attenuated and two virulent strains of H. capsulatum. In culture, the transition from the mycelial to the yeast form of all three strains can be triggered by a shift in temperature from 25° to 37°. Three distinct stages in the morphological transition of the attenuated strain have been delineated. Stage 1, immediately following the temperature shift, is characterized by rapid decline in ATP levels, uncoupling of oxidative phosphorylation and the induction of several heat shock proteins. After 24-40 hours, the cells are in a dormant period (Stage 2) which lasts 4-6 days. Stage 2 cells are characterized by very low rates of respiration, grossly decreased concentrations of mitochondrial election transport components, and low rates of protein and RNA synthesis. Stage 3 is characterized by increasing concentrations of cytochrome components, resumption of normal respiration, and induction of the yeast phase-specific enzymes. The 25 to 37°C transition of the virulent strains is similar, but the changes occur more rapidly and are less extreme. At higher temperatures (39-40°C), the morphologic transition of virulent strains is equivalent to the attenuated strain at 37°. Conversely, the behavior of the attenuated strain at 34° is equivalent to that of the virulent strain at 37°. Our results are consistent with the idea that a heat-related insult (uncoupling of oxidative phosphorylation and decline in ATP concentrations) triggers the morphological transition in all strains. The greater temperature sensitivity of the attenuated strain may be a key factor accounting for its decreased virulence.

1641 DIFFERENTIATION ASSOCIATED WITH SCLEROTIAL FORMATION IN PSYCHROPHILIC FUNGI. W. J. Newsted and N. P. A. Huner, Univ. of Western Ontario, London, Canada N6A 5B7.

The vegetative hyphae, sclerotial initials and mature sclerotia of four psychrophilic fungi, Myriosclerotinia borealis, Coprinus psychromorbidus, Typhula idahoensis and Typhula incarnata were studied biochemically and ultrastructurally. Cultures of these fungi were grown in darkness at 12°C on an agar medium until vegetative hyphae, sclerotial intitials and mature sclerotia were formed. Polypeptide complements of these structures were examined by 1D and 2D SDS-PAGE. Results indicated the presence of major low molecular weight polypeptides (12-30 Kd) in the sclerotia of all four fungi. The number and molecular weights of the major polypeptides varied from species to species. The major polypeptides constituted about 80 to 90% of the sclerotial polypeptide complement. These major polypeptides were absent or less conspicuous in vegetative hyphae and sclerotial initials.

In addition, vegetative hyphae, sclerotial initials and mature sclerotia of each fungus were examined by light and transmission electron microscopy (TEM). Thick sections were stained for protein, lipid and carbohydrate. Light microscopical analysis showed the presence of copious protein bodies in the cytoplasm of sclerotial cells of C. psychromorbidus, T. idahoensis and T.incarnata. Smaller proteinaceous inclusions were seen in the cytoplasm of sclerotial cells of M. borealis. The cells of vegetative hyphae and sclerotial initials were generally devoid of protein bodies but contained either amorphous proteinaceous material or small protein inclusions. TEM observations confirmed these results. Thus, the results indicate the accumulation of major polypeptides associated with differentiation of vegetative hyphae into sclerotial tissue in these psychrophilic fungi. However, microscopical results showed a variation in the packaging of these proteins in the species examined.

1642 β(1->3) GLUCAN SYNTHASE MUTANTS OF NEUROSPORA CRASSA. David R. Quigley and Claude P. Selitrennikoff, University of Colorado Health Sciences Center, Denver, CO 80262

β(1—>3) glucan synthase activity is essential for normal cell wall formation and is a key morphological determinant. The enzyme synthesizes β(1—>3) glucan, a structural cell wall polysaccharide, and is uncompetitively inhibited by sorbose. β(1—>3) glucan synthase activities of Neurospora crassa sorbose resistant mutants were investigated and the activities from two of the mutants were found to be more resistant to sorbose than wild-type. Two other mutant enzyme activities were noncompetitively inhibited by sorbose. β(1—>3) glucan synthase activities of the four mutants were characterized and compared to wild-type.

1643 DNA METHYLATION IN NEUROSPORA: CHROMATOGRAPHIC AND ISOSCHIZOMER EVIDENCE FOR CHANGES DURING DEVELOPMENT, P. J. Russell, K.D. Rodland, J.E. Cutler, E.M. Rachlin and J.A. McCloskey, Reed College, Portland, OR 97202; Montana State University, Bozeman, MT 53717; and Univ. of Utah, Salt Lake City, UT 84112.

The use of gas chromatography-mass spectroscopy to analyze the genomic DNA of the fungus Neurospora crassa clearly demonstrated the presence of small but measurable amounts of 5-methylcytosine (m^5Cyt), less than 0.300% relative to total base content. The m^5Cyt level appears to be developmentally correlated, as the vegetative spores or conidia contain 15 to 40% more m^5Cyt than actively growing mycelia. Isoschizomer analysis of the ribosomal RNA genes (the rDNA) using the enzymes MspI and HpaII confirmed the presence of m^5Cyt in both conidial and mycelial rDNA. The methylated cytosines are not randomly distributed throughout all CCGG sequences within the rDNA repeat unit, but are clustered specifically within the non-transcribed spacer region of the repeat unit. At least one of these sites appears to be methylated in conidia but not mycelia. [Supported by grants from NIH GM22488, GM26892, GM21584, and the American Cancer Society NP-441.]

1644 EXPRESSION OF THE cAMP-DEPENDENT PROTEIN KINASE DURING DEVELOPMENT OF D. DISCOIDEUM, K. L. Schaller*†, B. H. Leichtling* and H. V. Rickenberg*†, *National Jewish Hospital & Research Center and †Univ. of Colorado School of Medicine, Denver, CO 80206.

A function of cAMP in the overall development and morphogenesis of D. discoideum is well established and a role in cellular differentiation seems likely. Both the regulatory and catalytic subunits of the cAMP-dependent protein kinase (cA-d PK), a potential mediator of the effects of cAMP, are found in vegetative amoebae, and the levels of the subunits increase in parallel approximately five-fold during development. The increase represents de novo synthesis of the protein.

A large number of differentiation-specific mRNAs and proteins are found in either the prespore or prestalk cells of the pseudoplasmodia; cAMP regulates the accumulation of many of these mRNAs and proteins. We have shown that prespore cells have approximately four times as much cA-d PK as do prestalk cells. The levels of translatable mRNA for the regulatory subunit are being determined using antisera raised against the regulatory subunit in in vitro translation assays as a means of studying the mechanism by which the kinase is accumulated preferentially in prespore cells.

1645 AN EXTRACELLULAR PROTEIN IN THE BEAN RUST FUNGUS IS INVOLVED IN STOMATAL RECOGNITION, Richard C. Staples[1], Lynn Epstein[1], Lucille Laccetti[1], and H.C. Hoch[2], [1]Boyce Thompson Institute, Cornell University, Ithaca, NY 14853 and [2]New York Agricultural Experiment Station, Cornell University, Geneva, NY 14456

The bean rust fungus [Uromyces appendiculatus (Pers.) Unger] has a contact-sensitive mechanism to recognize the stomata, the fungus' penetration site on a leaf. We have used scratches on a polystyrene surface as a model for stomata; recognition is followed by mitotic nuclear division and the differentiation of the first infection structure, the appressorium. Our evidence suggests that an extracellular protein (or glycoprotein) is involved in stomatal recognition. Nuclear division of germlings incubated on scratched surfaces was significantly reduced by 500 µg/ml of either Pronase E (a nonspecific protease) or trypsin. Nuclear division was not affected by heat denatured Pronase E or trypsin, trypsin mixed with trypsin inhibitor or 500 µg/ml α- or β-glucosidase, α-mannosidase, or lipase. The time when germlings were sensitive to Pronase E corresponded to the time when the germlings were responsive to the thigmotropic stimulus. Pronase E did not effect germination but both adhesion and hydrophobicity of the germlings were significantly reduced. This suggests that extracellular matrix (ECM) proteins may be involved in hydrophobic bonding to an inducive surface. We speculate on two conceivable scenarios for the role of the ECM protein and the cytoskeleton in recognition: 1) The ECM protein(s) bind the germling to the surface so that the cytoskeleton can sense the inducive topography or 2) the ECM protein(s) bind and recognize the inducive topography, and then transmit the signal to differentiate to the cytoskeleton.

1646 INHERITANCE OF DNA METHYLATION IN COPRINUS CINEREUS, Miriam E. Zolan* and Patricia J. Pukkila†, *Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109 and †Department of Biology, University of North Carolina, Chapel Hill, NC 27514

In contrast to the nuclear genomes of many other fungi, that of the basidiomycete Coprinus cinereus is extensively methylated at the nucleotide doublet CpG. A centromere-linked, single copy locus was identified that is methylated in one geographical isolate of Coprinus and not in others. Tetrad analysis of this locus showed that methylated and unmethylated tracts are inherited 2:2 during meiosis, as expected for semiconservative replication of methylation. Surprisingly however, the methylation patterns of these tracts in meiotic progeny were often different from that of the methylated parent. To determine whether this meiotic variation is due to a special mechanism of inheritance of DNA methylation during meiosis, or whether it reflects mitotic variability of DNA methylation patterns, we examined veil cell isolates (mitotic products) and basidiospore isolates (meiotic products) from the same fruiting bodies. In both cases, 25% of the isolates had altered patterns of DNA methylation. Therefore, changes in DNA methylation are not confined to meiosis, but may occur during vegetative growth and/or fruiting body differentiation. Preliminary studies indicate that there is also extensive variation in methylation of the ribosomal RNA gene cluster. Since the ribosomal RNA genes are not linked to the other locus studied, changes in DNA methylation patterns may be widespread in the Coprinus genome. Most of the observed changes have involved increases in DNA methylation. Current studies are focused on the stability of these higher levels of methylation, and on relationships between DNA methylation and meiotic chromosome behavior. Supported by NSF (PCM8215694) and NCI (NRSA 5 F32 CA 07395).

1647 USE OF CLONED PROBE FOR ESTIMATING FUNGAL WILT DISEASE SEVERITY (IN LEMON) UNDER FIELD CONDITIONS, William V. Zucker, Isabella Di Silvestro, Nunziatella Butera, Agriculture Industrial Development S.p.A., Catania, Italy.

A major problem in understanding the extrinsic and intrinsic factors responsible for pathogen invasiveness under field conditions depends on accurate assessments of fungal growth. Determining this growth in wilt pathogens restricted to the host xylem tissue is particularly difficult and tedious; it is almost never quantitative. Through the use of cloned DNA from the pathogen (Phoma tracheiphila), we have been able to develop an extremely sensitive assay, using "spot hybridization" techniques to monitor the severity of the "mal secco" disease of lemons in Sicilian orchards. We have shown that the assay is quantitative and preferred to the more laborious, older methods. Utilization of this new method now allows us to study the etiology of the disease, efficacy of fungicide applications, and natural host defense mechanisms. Example will be shown.

Molecular Bases of Fungal Pathogenicity to Plants

1648 PHYTOTOXINS AS MOLECULAR DETERMINANTS OF PATHOGENICITY AND VIRULENCE, David G. Gilchrist, Department of Plant Pathology, University of California, Davis, CA 95616

Extracellular phytotoxins produced by plant pathogenic fungi are known to be causally involved in many plant diseases. Their role in disease extends from being uniquely required to incite disease (pathogenicity factor) to situations where infection can occur in the absence of the toxin but the toxin contributes to the severity of disease by influencing the relative virulence of the producing organism (virulence factor). Studies with both levels of toxin involvement have potential economic and scientific value. Disease resistant plant genotypes potentially may be identified from whole plants or cell cultures using toxins as screening tools. Toxins functioning as pathogenicity factors would be expected to select for high levels of resistance whereas an intermediate level would likely emerge from cells with differential sensitivity to a virulence determining toxin (1). Phytotoxins historically have been viewed as key chemical probes of mechanisms of disease induced plant stress as well as the molecular basis of genetically controlled disease avoidance (2).

Fungi in all classes have been shown to produce phytotoxins among which several species of Helminthosporium and Alternaria are particularly noteworthy. Evidence obtained with AAL-toxins produced by Alternaria alternata f. sp. lycopersici specifically pathogenic on tomato indicates that the toxins are produced both in culture and in infected host tissue. The host reaction to both the pathogen and the toxin is controlled by the same gene. Only isolates of A. alternata which produce AAL-toxins are pathogenic on tomato and the toxins reproduce the typical disease symptoms. Current in vitro results suggest that the AAL-toxins differentially interact with aspartate carbamoyl transferase (ACTase) in the respective host genotypes to effect a AAL-toxin:UMP synergistic inhibition. Alteration in regulation around the key ACTase step of pyrimidine biosynthesis with potential integrated metabolic disruption through interlocking pathway loops indicates a number of testable mechanisms to explain cell death due to AAL-toxins.

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1649 MECHANISM OF FUNGAL PENETRATION OF THE DEFENSIVE BARRIERS OF PLANTS, P. E. Kolattukudy, C. L. Soliday and C. P. Woloshuk, Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340.

Plant cuticle is a major barrier which fungal pathogens need to penetrate to infect plants (1). Cutin, a polyester composed of hydroxy and hydroxyepoxy fatty acids, is the structural component of the cuticle and therefore constitutes the major mechanical barrier to penetration. An extracellular fungal enzyme, cutinase, is involved in this process. This enzyme has been purified from many fungal pathogens (2). Since the ability of fungi to produce the enzyme can be a significant factor in determining the pathogenicity, the biosynthesis of the enzyme was studied. Under starvation conditions, cutin hydrolysate induced cutinase production in fungal cultures. mRNA was isolated from such cultures and cDNA was prepared and the cloned cDNA was completely sequenced (3). Restriction analyses of genomic DNA samples from two strains of Fusarium solani pisi, which differ markedly in their ability to produce cutinase, were done with ^{32}P labeled cDNA as the probe. Both contained one copy in identical size fragments. The high virulence strain which produces large amounts of cutinase contained a unique copy at a different location. Genomic DNA, cloned in λphage was sequenced revealing the presence of two introns in the gene. The structure and regulation of this gene will be discussed. Regulation of cutinase production by the spores in the early phases of germination is likely to be the critical factor in penetration of plants. Dot blot analysis with a labeled cDNA probe showed that within 15 to 30 min after the spores were exposed to cutin transcription of cutinase gene became measurable. Presence of cutin, its hydrolysate, or isolated monomers was necessary to induce transcription of cutinase gene. Thus, the spore is able to sense that it is on a plant surface before it initiates transcription needed for penetration.

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1650 GENETIC DETERMINANTS OF HOST SPECIFICITY IN A FUNGAL PLANT PATHOGEN. B. Valent, C.G. Weaver, K.A. Parsons, and F.G. Chumley. Department of Chemistry, University of Colorado, Boulder, Colorado, 80309. The major research objective of this laboratory is to determine the genetic and biochemical basis for host specificity in the fungal plant pathogen, Magnaporthe grisea (anamorph Pyricularia oryzae Cav. or Pyricularia grisea). M. grisea is a filamentous heterothallic Ascomycete that grows on defined medium. Conidia (asexual spores) of M. grisea initiate lesions on leaves, nodes and panicles of susceptible grasses. Individual isolates of the fungus exhibit a limited host range, infecting one or at most a few grass species. Isolates that cause blast disease of rice (Oryza sativa) are subdivided into hundreds of races according to the spectrum of rice cultivars they can infect. Field isolates of the pathogen with different host ranges are interfertile, permitting genetic analysis of host range determinants, although such studies have been hampered by low fertility. Field isolates of M. grisea vary in fertility according to host range. Isolates pathogenic to goosegrass and weeping lovegrass are moderately fertile, but rice pathogens show very poor fertility. We have initiated a program with the goal of improving the power and sophistication of genetic analysis of M. grisea. This includes the development of fertile laboratory strains that retain interesting pathogenicity characteristics. We are also working to develop a genetic transformation system in order to clone M. grisea genes that control pathogenicity and virulence. We have accumulated a collection of over 200 mutants, including nutritional, morphological and drug resistant variants. We have characterized heterokaryons and diploids of M. grisea in order to use these forms of the fungus in conducting genetic analysis.

Genetic crosses between field isolates of the fungus already have yielded insights into the basis for fertility and pathogenicity, and they have defined single genes that determine certain aspects of host specificity. We have characterized two genes that encode enzymes important for polyketide synthesis. Mutations in these genes lead to abnormal buff or white pigmentation of the mycelium, and they cause a total loss of pathogenicity. We have also defined two genes that determine the ability of pathogenic isolates to infect weeping lovegrass or goosegrass. We are especially interested in characterizing and cloning hypothesized avirulence genes that determine incompatibility with cultivars of rice that carry corresponding blast resistance genes. An understanding of how these genes function to determine the outcome of a challenge by the pathogen will yield insights into methods for incorporating stable disease resistance into crop plants. [Supported by the USDOE (DE-AC02-84ER13160), by the USDA (83-CRCR-1-1263) and by Monsanto Agricultural Products Company.]

1651 VIRULENCE REGULATION BY CYTOPLASMIC ELEMENTS, Neal K. Van Alfen, Department of Biology, UMC-45, Utah State University, Logan, UT 84322

The expression of virulence by fungal plant pathogens is a manifestation of considerable evolutionary specialization. The mechanisms of this expression are generally poorly understood. Traditionally, the approach to elucidating the nature of virulence has been to seek easily selectable products responsible for plant cell stress. The discovery of cytoplasmic elements that can regulate fungal virulence (1 & 2) has provided an opportunity for new approaches to the study of this problem. In the fungus Endothia parasitica, cytoplasmic genes have been demonstrated to modulate the expression of virulence (1). In some cases, the reduction in virulence expression (hypovirulence) is transmissible to other strains of the fungus. Double-stranded RNA has been correlated with the presence of this transmissible hypovirulence (3). The dsRNA, a multi-segmented genome, is not encapsulated in a protein coat like most viruses; it is packaged within fungal vesicles. Strains showing non-transmissible hypovirulence can be generated readily by growth of some wild-type strains on hypertonic media. In these strains, the susceptibility to phenotypic modification by osmotic shock appears to be a cytoplasmic character. At least one of the loci whose expression is affected by hypertonic media is nuclear. Current investigations are directed toward the hypothesis that cytoplasmic gene(s) affect the expression of nuclear genes, some of which may be involved in virulence, and toward the elucidation of similarities between transmissible and non-transmissible hypovirulence.

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