MOLECULAR AND CELLULAR BIOLOGY OF YEASTS AND FILAMENTOUS FUNGI

Organizers: William Timberlake and Michael Holland

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Plenary Sessions Page

Poster Session

Chromosome Sructure and Function-I

N 001 MOLECULAR ASPECTS OF MEIOTIC PAIRING IN SACCHAROMYCES, Breck Byers, Nancy Hollingsworth and Loretta Goetsch, Department of Genetics, University of Washington, Seattle, WA 98195

The generation of viable meiotic products in Saccharomyces cerevisiae and many other species generally depends on premeiotic pairing and recombination because non-recombinant chromatids nondisjoin in the reductional division. The demonstration by Easton-Esposito and her colleagues that spo13 strains (which undergo only a single division that is largely equational) yield viable ascospores in the absence of recombination[1], provides a method for identifying mutations that affect pairing and exchange. Using this system, we have isolated and characterized the gene HOP1, which is essential for the normal level of crossing-over but is dispensible for intrachromatid recombination and/or unequal sister chromatid exchange[2]. Screening a library for plasmids that complemented the spore inviability shown by hop1 SPO13 diploids yielded the HOP1 clone, which was sequenced and used for analysis of transcription. The derived amino acid sequence represents a protein of 606 residues containing a segment similar to the "zinc-finger" motif of certain nucleic acid-binding proteins. Site-directed mutagenesis has shown that the motif is essential for function[3]. Northern blots demonstrate a transcript that is present only during meiosis, consistent with the viability of deletion strains[3]. Affinity-purified antiserum directed against a trpE-HOP1 fusion protein has revealed the presence of the HOP1 product within meiotic nuclei. Electron microscopy with a colloidal gold-labeled probe demonstrates that the protein resides within the synaptic structure of the pachytene bivalents. Although chromatography is hindered by insolubility of the gene product, binding to DNA is evident. We suggest that the HOP1 product plays a direct role in chromosomal pairing for meiotic crossing-over. (Supported by NIH grants GM18541 and GM07735.)

[1]Malone, R.E., and R.E. Esposito, 1981 Mol. Cell. Biol. 1, 891-901; Wagstaff, J.E., S. Klapholz, and R.E. Esposito, 1982 Proc. Natl. Acad. Sci. USA **79**, 2986-2990.
[2]Hollingsworth, N.M., and B. Byers, 1989 Genetics **121**, in press.
[3]Hollingsworth, N.M. 1988 Dissertation, Univ. of Washington.

N 002 CENTROMERES AND FUNCTIONAL MINICHROMOSOMES IN BUDDING AND FISSION YEASTS, Mary Baum, Karen Hahnenberger, Hans Lechner, Francois Perier, Louise Clarke and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, CA 93106

The functional kinetochores in chromosomes of budding yeast (Saccharomyces cerevisiae) are relatively small, consisting of 220-250 bp of centromere (CEN) DNA folded into a chromatin particle bounded by hyper-sensitive nuclease cleavage sites (1). The consensus CEN sequence (about 110-120 bp in length) contains a key protein binding site termed CDEIII (TGT(T/A)T(T/A)TG.TTTCCGAAA....AAA). Chromatin protein(s) binding specifically to wild-type, but not mutationally inactivated CDEIII DNA sequences have been detected by fragment mobility shift assays (2). The CDEIII-binding protein must be phosphorylated for binding to occur. A combined molecular and genetic approach is being used to identify yeast genes necessary for synthesis of functional CDEIII-binding proteins.

In contrast, the functional centromere regions on the three chromosomes of the fission yeast Schizosaccharomyces pombe are relatively large, containing 50-80 kb of moderately repetitive untranscribed DNA sequences (3,4). These centromere DNAs have been cloned on yeast artificial chromosome (YAC) vectors in both budding and fission yeast. In S. pombe, synthetic linear or circular minichromosomes containing an intact S. pombe centromere are stable mitotically and segregate properly through meiosis (5). The smallest functional centromeric DNA sequence thus far identified in S. pombe is about 40 kb in length. The budding and fission yeast centromeres are not interchangeable; neither functions in the heterologous cell. The relatively large kinetochores seen in fission yeast are more typical of what has been observed in most eukaryotes. The use of YAC vectors to clone large DNA fragments in yeast presents a general strategy for the isolation of functional centromeres from a wide variety of eukaryotic cells.

1. Reviewed in L. Clarke and J. Carbon, Ann Rev. Genet. 19, 29-56 (1985).
2. R. Ng and J. Carbon, Mol. Cell. Biol. 7, 4522-34 (1987).
3. Y. Nakaseko et al., EMBO J., 5, 1011-21 (1986).
4. B. Fishel et al., Mol. Cell. Biol. 8, 754-63 (1988).
5. K. M. Hahnenberger et al., Proc. Natl. Acad. Sci. USA, in press.

N 003 CHROMOSOME STRUCTURE AND REGULATION IN YEAST, Gerald R. Fink, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA, 02142 and Department of Biology, MIT, Cambridge, MA 02139. The avidity of the homologous recombination system in *Saccharomyces cerevisiae* has a number of biological consequences. Exact duplications created artificially by transformation are lost at high frequency both in mitosis and meiosis via homologous recombination. Yet, many natural duplications survive. For example, short duplications of ~10-20 bp that function as regulatory sequences are found upstream of many genes. These repeats are the targets for trans-acting regulatory proteins and their retention suggests some immunity from the recombination system. The ribosomal RNA is encoded by ~200 tandem repeats of a 10 kb unit located on chromosome XII. Previous studies have shown that homologous recombination in the rDNA is suppressed during meiosis. We have found that mitotic recombination in the rDNA cluster is suppressed by DNA topoisomerases I and II. Strains containing mutations in either *TOP1* or *TOP2* have a rate of mitotic recombination in the rDNA approximately 100 fold higher than do wild type strains. These mutations have little, if any, effect on recombination at other loci we tested. Thus, topoisomerease I is required to prevent this high level of recombination in the rDNA repeats. Chromosome XII in a top1 strain shows aberrant migration on pulsed field gels, whereas all the other chromosomes show the same migration as they do in a *TOP1* strain. The aberrant migration of chromosome XII is observed only in DNA preparations made from *top1* cells in log phase; chromosome XII migrates normally in DNA prepared from cells in stationary phase.

N 004 DNA METHYLATION AND GENETIC INSTABILITY IN NEUROSPORA CRASSA. Eric U. Selker, Institute of Molecular Biology, University of Oregon, Eugene, OR, 97403 A novel genetic mechanism ("RIP") inactivates duplicated genes in Neurospora crassa. RIP operates in the period between fertilization and karyogamy in the life cycle. The process is remarkably efficient--even unlinked duplicated genes are altered at high frequency. Although both copies of a duplication are affected, a cell can survive duplication and inactivation, even of an essential gene, since RIP is nucleus-limited, and operates only in cells having a nucleus from each parent. Alteration of the DNA sequence of duplicated regions was first inferred from Southern hybridizations showing dramatic changes ("rearrangement") in the pattern of restriction sites. We now know that RIP does not result in classical rearrangements (deletions, insertions, inversions, etc.) Rather, RIP results in extensive point mutations of a specific type: G·C pairs change to A·T pairs (RIP = repeat induced point mutation). The existence of RIP and the nature of the resulting alterations have important implications for evolution. Furthermore, this demonstration that cells can "cause" mutations prompts us to reexamine the meaning of "spontaneous" mutation. An interesting feature of the RIP process is that it typically results in methylation of cytosines in the affected sequences. DNA methylation may both play a role in the mechanism of RIP and be a consequence of the process. In effect, RIP creates "signals" for de novo DNA methylation by the simple substitution of some G·C pairs by A·T pairs. This conclusion may be an important clue for solving the mystery of what causes methylation in eukaryotes.

Chromosome Structure and Function-II

N 005 DNA REPLICATION IN *SACCHAROMYCES CEREVISIAE*. Judith L. Campbell, Martin E. Budd, Colin Gordon, Peter Rhode, and Karen C. Sitney, Divisions of Biology and Chemistry, California Institute of Technology, Pasadena, California 91125.

We have used yeast genetics and biochemistry to define the functions of the numerous DNA polymerases found in yeast. Many years ago we showed that DNA polymerase I, the analog of metazoan DNA polymerase α, was essential for DNA replication in yeast. More recently we have carried out structure function studies of DNA polymerase I. We have shown that a protein with a 155 amino acid deletion at the N-terminus of the protein is able to complement all functions of DNA polymerase I *in vivo*. By contrast, a 250 amino acid deletion from the C-terminus (nucleotide residues 1214-1468) results in a nonfunctional DNA polymerase. Five temperature sensitive mutations, described previously, all map to conserved regions II and III in the DNA polymerase.

We have recently found that there is a second essential DNA polymerase in yeast, DNA polymerase III. This was shown by demonstrating that yeast *cdc2* mutants, which are deficient in replication, are deficient in DNA polymerase III. We have cloned the *CDC2* gene and are studying the regulation of the polymerase.

We have purified DNA polymerase II to apparent homogeneity. The protein has a molecular weight of 170 kDa on activity gels. The polymerase activity copurifies with a 3′→5′ exonuclease through all purification steps. The preferred template is poly(dA):oligo(dT), suggesting that the enzyme is more processive than either DNA polymerase I or DNA polymerase III. DNA polymerase II is sensitive to aphidicolin and resistant to 100 μM BuPdGTP; thus it is not a member of the polymerase β family. Two proteins that stimulate DNA polymerase II have been identified, one of which increases the processivity of polymerase II. DNA polymerase II is not encoded by *POL1*, *CDC2*, or *REV3*, three genes that encode yeast DNA polymerases. On the basis of its biochemical characteristics, we propose that DNA polymerase II is analogous to the delta polymerases and may be involved in repair.

We have observed a fourth DNA polymerase. Since this species differs from polymerase II only in chromatographic properties, we have not yet given the enzyme a new designation.

In addition to our work with DNA polymerases, we have investigated protein DNA interactions at the yeast origin of replication, *ARS1*. We have purified ABF1, a protein that binds within 100 base pairs of the core consensus of at least seven different ARS elements. The protein shows two species of 132 and 135 kDa. Antibodies have been used to clone the *ABF1* gene and gene disruptions show that *ABF1* product is essential for the viability of yeast. The DNA binding domain has been identified by deletion analysis.

N 006 REGULATION OF TRANSCRIPTION BY HISTONES AND NUCLEOSOMES IN THE YEAST, SACCHAROMYCES CEREVISIAE, Michael Grunstein, Lianna Johnson, Linda Durrin, Paul Kayne and Ung-Jin Kim, Molecular Biology Institute and Department of Biology, University of California, Los Angeles 90024

Two means by which histones or nucleosomes regulate transcription will be presented. In the first, point mutations affecting positively charged amino acids of the histone H4 N-terminus have been shown to cause specific activation of the silent mating loci, *HMLalpha* and *HMRa*. We are using these point mutations in an attempt to identify second site revertants in proteins which interact with histone H4 to regulate the silent mating loci. In the second approach, we have previously shown that nucleosome loss, caused by glucose repression of a *GAL1* promoter-histone H4 fusion gene, leads to activation of transcription. This occurs for at least three different genes (galactokinase [*GAL1*], cytochrome c [*CYC1*] and acid phosphatase [*PHO5*]). Activation of transcription by nucleosome loss requires only the downstream promoter elements (TATA and initiator sequences). We have recently extended the generality of this mode of activation to other regulated genes and are attempting to determine the mechanism by which nucleosome loss activates transcription initiation.

N 007 GENE ACTIVATION AND TRANSCRIPTION IN YEAST, Roger D. Kornberg, Andrew R. Buchman, Daniel I. Chasman, Aled M. Edwards, Peter Flanagan, Raymond Kelleher, Yahli Lorch, and Neal F. Lue, Department of Cell Biology, Stanford University, School of Medicine, Stanford, California 94305.

Multiple DNA-binding factors mediate effects of UAS_G on transcription. One factor creates a nucleosome-free region containing binding sites for the second factor, GAL4 protein. Purified GAL4 protein is an oligomer, with subunits of GAL80 protein, which regulate GAL4 action. A number of transcriptional activators, including a fusion of GAL4 to herpes VP16, and a yeast factor variously termed GRFI, TUF, and RAP1, stimulate transcription by yeast RNA polymerase II in an in vitro system. The components of this system have been resolved, and the mechanism of activation has been pursued with purified components.

Reproductive Strategies-I

N 008 TEMPORAL PROGRAM OF GENE EXPRESSION DURING SPORULATION IN SACCHAROMYCES CEREVISIAE, Jacqueline Segall, Anthony Percival-Smith, David T.S. Law, Peter Briza, and Michael Breitenbach*. Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8 and * Institut fur Mikrobiologie und Genetik, Universitaet Wien, Althanstrasse 14, A-1090 Wien, Austria.

The sporulation process in *S. cerevisiae*, restricted to diploid *MATa/MATα* cells, consists of meiosis followed by the encapsulation of the haploid nuclei within spore walls. Using a differential hybridization screening procedure, we have identified two temporally distinct classes of sporulation-specific (*SPS*) genes. Fourteen middle *SPS* genes and two late *SPS* genes have been characterized. The middle *SPS* genes are first expressed at 6 to 8 h after transfer of cells to sporulation medium with maximal transcript accumulation occurring at 8 - 10 h, the time at which meiosis is nearing completion; transcript levels then decrease with almost no transcripts being detectable at 20 h. The late *SPS* genes are activated at 10 - 12 h with maximal transcript accumulation occurring at 15 - 20 h, a time coincident with spore wall formation.

Mutation of the *SPS1* gene, a middle gene, has demonstrated that its product is essential for completion of the sporulation process. Cells of a *MATa sps1/MATα sps1* strain become tetranucleate on transfer to sporulation medium but spore walls do not form. The predicted properties of the protein encoded by the *SPS100* gene, a late gene, suggest that it is a component of the spore wall; the protein contains a potential signal sequence and cleavage site and numerous sites for potential glycosylation. Cells of a *MATa sps100/MATα sps100* strain are indistinguishable from cells of the wild-type strain when assessed for efficiency of ascus formation and spore viability. However, the onset of resistance to ether, a property of mature spores, is delayed by 5 h in the mutant strain. We presume that this defect represents a delay in spore wall maturation. These studies confirm that the products of genes identified on the basis of a development-specific expression pattern contribute to the sporulation process.

We have recently found that dityrosine is a major component of the outer layer of the spore wall. Taking advantage of the natural fluorescence of dityrosine we have isolated 8 mutants that have greatly reduced levels of dityrosine in the spore wall. By screening a genomic library for complementation of this defect, we have found that 6 of the mutants represent 2 complementation groups. The corresponding genes are sporulation-specific, contiguous, and expressed at the time at which dityrosine accumulates.

Reproductive Strategies-II

N 009 OPERATION OF THE NITROGEN AND SULFUR REGULATORY CIRCUITS OF NEUROSPORA CRASSA, George A. Marzluf and Ying-Hui Fu, Department of Biochemistry, Ohio State University, Columbus, OH 43210

In both the nitrogen and sulfur circuits, a major positive-acting control gene is responsible for turning on an entire set of structural genes. The cys-3 regulatory gene encodes a protein of 236 amino acid residues which activates the expression of S-related genes; this protein contains a putative leucine zipper DNA-binding element, whose function has been implied by site-directed mutagenesis studies. The DNA recognition site for the cys-3 protein has been identified by use of gel band mobility shift experiments and DNA footprinting. In the nitrogen control circuit, a major regulatory gene, designated nit-2, turns on the expression of various nitrogen-related structural genes. The nit-2 gene encodes a regulatory protein of approximately 105,000 daltons and contains a possible zinc finger element. Both the positive-acting nit-2 regulatory gene and a negative-acting regulatory gene, nmr, are involved in nitrogen catabolite repression. Deletion mutants which lack the carboxy terminus of the nit-2 protein still activate the expression of the nitrate reductase gene but are not subject to nitrogen repression. The nmr protein may bind to the carboxy terminus of the nit-2 protein to inhibit the activation function of nit-2.

N 010 MATING TYPE IN NEUROSPORA: IT WOULD RATHER FIGHT THAN SWITCH, Robert L. Metzenberg, N. Louise Glass, Jeff Grotelueschen, and Mary Anne Nelson, Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706. Neurospora crassa is heterothallic -- sexual fusion, formation of fruiting bodies (perithecia) and progress through meiosis and sporulation occurs only when individuals of the two mating types, A and a, are brought together on a nutrient-limited medium. The two mating types are specified by alleles of a single gene. On medium which is not nutrient-limited, the behavior is the opposite: only like mating type strains can fuse to form a stable heterocaryon, whereas fusion of opposite mating types results in cell death. Thus the mating type alleles have one set of functions during the sexual cycle, but act as heterocaryon incompatibility loci during vegetative growth. Mutants selected for vegetative compatibility are usually sterile (1). Unlike in the yeasts, switching from one mating type to the other has never been seen.

Cloning of the two mating type alleles (2) has opened the system to molecular analysis. Surprisingly, the two alleles are non-homologous over a distance of about 4.4 kbp (3). In addition, there is only one copy per genome, and unlike in yeast, there is no inactive copy of the opposite mating type allele. Switching, then, is impossible because information for the opposite mating type is absent. There are, however, homothallic species in the genus Neurospora and in closely related genera, and these fall into two groups. One contains only the A allele, which can somehow function in the sexual cycle without a partner. The other group contains both A and a, and in this latter group, facile switching is at least a possible basis for homothallism.

Progressive exonucleolytic deletion of the DNA of the A mating type allele followed by transformation of a sterile mutant with the truncated DNA has defined the region needed for fruiting body formation, and has also shown that it is coextensive with the region which confers heterocaryon incompatibility.

The functionally important sequences do not contain obvious DNA binding motifs, but we believe that the mating type alleles must cooperate to promote the transcription of target genes specific to the sexual cycle. Recently we have isolated DNA sequences which are transcribed on nutrient-limited medium appropriate to mating, but not on medium appropriate for vegetative growth. Most of these are not transcribed in sterile mutants derived from A strains, even in nutrient-limited medium. Some properties of these putative targets of the mating type alleles will be discussed.

(1) Griffiths, A.J.F. (1972) Can. J. Genet. Cytol. 42: 167. (2) Vollmer, S.J. and Yanofsky, C. (1986) Proc. Nat. Acad. Sci. U.S.A. 83:4869. (3) Glass, N.L., Vollmer, S.J., Staben, C., Grotelueschen, J., Metzenberg, R.L. and Yanofsky, C. (1988) Science 241:570.

N 011 THE Aα MULTIALLELIC MATING-TYPE GENES OF SCHIZOPHYLLUM COMMUNE. Robert C. Ullrich, Luc Giasson, Charles A. Specht, Mary M. Stankis and Charles P. Novotny, Departments of Botany and Microbiology, University of Vermont, Burlington, VT 05405. Sexual development in Schizophyllum is regulated by four multiallelic mating-type genes: Aα, Aβ, Bα and Bβ. There are nine Aα alleles in the worldwide population. We isolated two Aα alleles. Aα4 was isolated by walking the chromosome from PAB1 in a cosmid library made from an Aα4 strain. Aα1 was isolated by using the insert of an Aα4-containing cosmid to probe a cosmid library made from an Aα1 strain. The isolated cosmids activate A-regulated development when transformed into recipients carrying alternative Aα alleles. Mating-type transformants were screened microscopically for A-activated development and were confirmed by mating tests and genetic analyses of progeny. The identity of alleles was confirmed by Southerns and by transformations in which the activation of A-regulated development is dependent upon the specific Aα alleles of the recipient and the transforming DNA. Aα1 and Aα4 have been subcloned to 2.8 and 1.2 kb fragments respectively. When transformed into wild-type recipients, these short fragments activate all of the A-regulated developmental events. The transforming alleles are active in trans. Aα1 and Aα4 probes: hybridize only to Aα1 and Aα4 DNA respectively, do not hybridize to DNA from other Aα strains (even at low stringency), and do not hybridize to the DNA from Aβ, Bα or Bβ loci. The fact that there is only one Aα allele per haploid genome rules out models that postulate multiple alleles. Aα alleles map to the same region of the chromosome, but are embedded within a 4-6 kb region of heterogeneous, allele-specific sequence. The structure, sequence and functional domains of Aα1 and Aα4 will be discussed.

Control of Cell Proliferation

N 012 The *RAS* Pathway and Cell Cycle Control in *Saccharomyces cerevisiae*. James Broach, Stephen Garrett, Sara Jones, Mary Fedor-Chaiken, Shira Silberberg, Regine Hengge-Aronis, Robert Deschenes, Steve Clarke, and Jeff Stock. Department of Biology, Princeton University, Princeton, NJ 08544.

RAS proteins are required for initiation of the mitotic cell cycle in yeast, predominantly, if not exclusively, through their stimulation of adenylate cyclase and subsequent activation of the cAMP-dependent protein kinase (A kinase). *RAS* proteins are extensively postranslationally modified as part of the process of localization to the plasma membrane. We have shown that, in addition to becoming acylated, *RAS* proteins are proteolytically cleaved to remove the terminal three amino acids and then methyl esterified on the free carboxyl terminus. We have identified a gene, *SRV2*, which, like *RAM1/DPR1*, is likely to be required to effect this maturation process. In order to become activated, *RAS* protein must bind GTP. Using an *in vitro* nucleotide exchange assay, we have obtained evidence that the product of *CDC25* activates *RAS* protein by catalyzing exchange of GDP bound to *RAS* protein for free GTP.

Most of the characterized intracellular targets of the A kinase are proteins involved in assimilation, storage and metabolism of carbohydrates. We have investigated whether cAMP also has a significant effect on patterns of transcription by searching for genes whose transcription is induced rapidly on refeeding cAMP to cAMP-starved cells. We identified 35 unique clones containing cAMP inducible transcripts. These genes are expressed at low levels in cells starved for cAMP, nitrogen or sulfur and are induced ten to fifty fold within thirty minutes of refeeding the starved cells, whether or not cell cycle progression is blocked by prior addition of mating factor. These genes define a class of growth specific genes, whose expression is uniform during proliferation but repressed in quiescence. These results suggest that quiescence in yeast represents a distinct state and is not simply an early event in the cell cycle. We propose that cAMP and nutrient regulation of transcription of these essential genes may play a central role in the regulation of mass accumulation by the cell and attendant initiation of the mitotic cell cycle.

By isolating revertants of *ras1 ras2*^{ts} strains, we have identified three novel loci in the RAS/cAMP pathway. One of these genes, *YAK1*, encodes a protein kinase whose inactivation suppresses not only loss of *ras* activity but also complete loss of A kinase activity. These results suggest that *YAK1* is either a critical target of the A kinase or that *YAK1* kinase and A kinase have antagonistic effects on protein targets directly involved in growth regulation.

N 013 THE REGULATORY CIRCUITRY FOR GLUCOSE REPRESSION IN YEAST,
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In S. cerevisiae a global regulatory system controls expression of many genes in response to glucose availability. Our studies of this regulatory system have focused on one glucose-repressible gene, the SUC2 (invertase) gene. Cis-acting elements in the SUC2 upstream regulatory region have been identified, and DNA-binding activities that recognize this region have been characterized. Trans-acting genes required for regulated expression of SUC2 (and other glucose-repressible genes) have been identified by isolating mutants defective in either glucose repression or derepression. Genetic analysis of the interactions among these mutations has led to a model for the regulatory circuitry.

Molecular analysis of the trans-acting genes has provided evidence for their functions. One of the genes required for expression of glucose-repressible genes, SNF1, encodes a protein kinase. Genetic evidence suggested a functional relationship between SNF1 and SNF4. Biochemical studies showed that the SNF4 product is required for maximal activity of the SNF1 protein kinase in vitro. In addition, a bifunctional SNF4-β-galactosidase fusion protein coimmunoprecipitates with the SNF1 protein kinase, suggesting physical association in vivo. Candidates for targets of the protein kinase were identified by selecting extragenic suppressors of a snf1 mutation. One of the genes identified in this way, SSN6, appears to encode a negative regulator of SUC2 and other genes. Null mutations in SSN6 cause constitutive SUC2 expression, whereas the presence of multiple copies of SSN6 reduces derepression of SUC2. A bifunctional SSN6-βgalactosidase fusion protein is localized to the nucleus, suggesting that the SSN6 protein acts as a fairly direct negative regulator. Three other genes required for derepression of SUC2 most likely play general roles in transcription as they also affect promoters that are not glucose-repressible; two of these (SNF2 and SNF5) encode nuclear-localized proteins.

N 014 SIGNAL TRANSDUCTION PATHWAYS REGULATING THE G1 PHASE OF YEAST CELL CYCLE,
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Saccharomyces cerevisiae has two different signal transduction systems operating in the G1 phase of the cell cycle. One is mediated by nutrients, such as glucose, which positively regulate the early G1 phase; the other is mediated by mating pheromones which negatively regulate the late G1 phase.

Mating pheromone pathway: GPA1, STE4 and STE18 comprise the respective α, β and γ subunits of the mating pheromone specific G protein heterotrimer, which couple the cell surface receptor to a putative intracellular effector. It is thought that in the absence of pheromone, the α subunit binds GDP and associates with the $\beta\gamma$ complex, inhibiting its activity. The binding of pheromone to its receptor promotes the exchange of the bound GDP with GTP, resulting in dissociation of the $\beta\gamma$ subunits from the GTP-bound α subunit. The released $\beta\gamma$ complex is thought to propagate the signal.

RAS-cAMP pathway: The RAS proteins play a key role in the transduction of the signal for growth in response to nutrients. The signal is transduced by virtue of the RAS proteins ability to bind and hydrolyze GTP. In the GTP-bound state, RAS activates adenylate cyclase. At least two proteins are involved in the regulation of the processes leading to the activation of adenylate cyclase. CDC25 has been proposed to mediate the nucleotide exchange on RAS, namely the exchange of GDP to GTP. IRA1 and IRA2, when disrupted, cause the formation of high levels of cAMP and suppression of the lethality of the CDC25 disruption. Therefore, they are possibly involved in the regulation of the intrinsic GTPase activity of RAS.

Transcriptional Control Mechanisms-I

N 015 COORDINATE REGULATION OF GLYCOLYTIC GENE EXPRESSION, Janice P. Holland, Paul Brindle, Catherine E. Willett and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616.

Maximal transcription of most yeast glycolytic genes requires the product of the regulatory gene *GCR*1. Transcription of the enolase genes *ENO*1 and *ENO*2, for example, is reduced 20 to 50-fold in a strain carrying a *gcr*1 null mutation. The *ENO*2 gene contains two adjacent UAS elements located approximately 460bp upstream from the transcription initiation site. *ENO*2 expression is not affected by deletion of either UAS element alone, however, removal of both abolishes transcription. Interestingly, deletion of the distal UAS element alone permits wild type levels of *ENO*2 transcription in a strain carrying the *gcr*1 mutation. These results show that the distal UAS element acts as a silencer in a *gcr*1 strain and an enhancer in a *GCR*1 strain. Proteins that bind to both *ENO*2 UAS elements were separated by phosphocellulose and Mono S chromatography. The protein which binds to the distal UAS is not encoded by *GCR*1, however, both the size of the protein complex formed with the distal UAS and the affinity of the binding protein for this site are strongly affected by the *GCR*1 gene product. Several lines of evidence show that the protein which binds the proximal UAS is the product of the general regulatory gene *RAP*1. The binding sites for the *RAP*1 protein and the protein which binds the distal UAS partially overlap, giving rise to mutually exclusive binding of the two proteins. The biological activity of a double-stranded oligonucleotide containing both *ENO*2 UAS elements was tested after insertion at sites upstream from the TATAAA boxes of *ENO*1 and *ENO*2 test genes. In all cases, the oligonucleotide served as an orientation independent UAS element, however, the UAS activity was not regulated by *GCR*1. The results, therefore, show that the distal UAS element in *ENO*2 is required for, but is not sufficient for, *GCR*1-dependent regulation of gene expression.

Binding sites for the *RAP*1 protein and the protein which binds to the distal UAS in *ENO*2 have been mapped within the 5' flanking regions of *ENO*1 and one of the glyceraldehyde-3-phosphate dehydrogenase genes (*TDH*3). Small deletion mutations within 5' flanking regions of *ENO*1 and *TDH*3 have also been identified which render their expression independent of *GCR*1. These results indicate that the mechanism of *GCR1*-dependent control of *ENO1, ENO2* and *TDH*3 is similar. We propose that the *GCR*1 protein modulates the enhancer/silencer functions of specific UAS elements within the 5' flanking sequences of yeast, glycolytic genes.

Transcriptional Control Mechanisms-II

N 016 MULTIPLE REGULATORY CIRCUITS IN Aspergillus nidulans, Michael J. Hynes, Meryl A. Davis, Alex Andrianopoulos, Timothy G. Littlejohn, Robyn van Heeswyck, Jennifer A. Saleeba, Ruth A. Sandeman, Margaret E. Katz and Imogen B. Richardson, Department of Genetics, University of Melbourne, Parkville, Victoria 3052, Australia. The amdS gene is subject to regulation by multiple independent control circuits mediated by defined regulatory genes. These control circuits are shared by sets of co-regulated genes. Analysis of the 5' sequence of the amdS gene in various in vivo and in vitro generated mutants has been used to define the sites of action of the various regulatory gene products. The amdR regulatory gene has been found to encode a zinc finger DNA binding domain in the N-terminal region of the protein and a C-terminal region necessary for activation of gene expression. Sequences involved in amdR binding have been identified in four genes regulated by omega amino acid induction. The amdR gene is expressed constitutively at a low level. In contrast the facB gene involved in acetate induction of amdS expression is regulated itself by acetate induction and carbon catabolite repression. At least one sequence involved in facB binding has been identified in the 5' controlling region of amdS and this is separate from the amdR binding sequence. Both of these sequences are fairly sensitive to spacing with respect to the amdS start site. GA rich sequences found 5' to both the amdS and aciA genes are implicated in regulation mediated by the amdA gene. Nitrogen metabolite control of the amdS gene and many other genes is mediated by the areA gene. Preliminary evidence indicates that there may be more than one site of action for this gene in the 5' region of amdS. A further control circuit has been identified by finding a protein that binds to a CCAAT motif and some in vivo evidence indicates that this sequence is important for setting the level of amdS expression. A CCAAT sequence is closely associated with the amdR binding site in both the amdS and gatA genes but the binding activity of these proteins appears to be independent.

Post-Transcriptional Control Mechanisms

N 017 RNA SPLICING IN SACCHAROMYCES CEREVISIAE: IN VITRO ANALYSIS OF GROUP II SELF-SPLICING AND GENETIC STUDIES OF PRE-tRNA PROCESSING.

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Four systems of RNA splicing coexist in the budding yeast, S. cerevisiae. Two systems are mitochondrial, Group I and Group II introns; both of these groups include self-splicing examples and are widely distributed in fungal mitochondria. The nuclear genes of this yeast include examples of both pre-mRNAs and pre-tRNAs with introns, likewise typical of fungal genes.

Group II introns, such as oxi3 intron 5-gamma (AI5γ), splice by a lariat-forming pathway. The intron AI5γ is self-splicing; the RNA is necessary and sufficient for the reaction to proceed in vitro. Interruption of the intron at certain sites does not prohibit splicing -- instead, the two half-molecules associate productively to carry out all regular reactions (K. A. Jarrell, et al., 1988, Mol. Cell Biol. 8, 2361). The ability of sections of the intron to reassociate provides a convenient assay for interaction between separate domains of the secondary structure and to identify various dispensable regions. Other domains are essential for specific steps of the reaction. The half-molecules from distinct introns can be interchanged to test for functional equivalence.

The standard model for pre-tRNA processing holds that terminal processing precedes splicing. Recently, we have analyzed in vivo RNA and identified apparent intermediates for an alternative pathway that allows splicing to precede terminal maturation. Splicing itself requires two separable activities -- an endonuclease and an RNA ligase. Several mutations identify functions required for pre-tRNA splicing. These include the RNA ligase; its gene was previously cloned and shown to be essential (E. Phizicky, et al., 1986, J. Biol. Chem. 261, 2978). At least two other genes have been implicated in specifying the endonuclease activity (M. Winey and M. Culbertson, 1988, Genetics 118, 609). We have found evidence for yet another gene, designated pta1 (for pre-tRNA accumulation), affecting pre-tRNA splicing. Our analysis shows that this mutant represents a new gene of the splicing pathway.

N 018 POST-TRANSLATIONAL MODIFICATION AND MITOCHONDRIAL IMPORT OF YEAST CYTOCHROME *C*,

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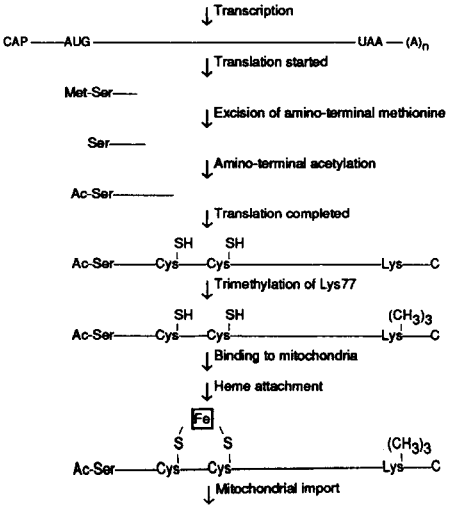
In addition to transcription and translation, the biosynthesis of mitochondrial cytochrome *c* includes the following post-transcriptional steps for formation and localization of the final product: excision of the amino-terminal methionine residue; amino-terminal acetylation of certain cytochromes *c*; trimethylation of specific lysine residues in fungal and plant cytochromes *c*; and the covalent attachment of protoheme to two cysteinyl residues, concomitant with transport into the mitochondrial membrane. Subsequently, mature cytochrome *c* interacts with a variety of other heme proteins to carry out various redox reactions. Mutationally altered forms of iso-1-cytochrome *c* from the yeast *Saccharomyces cerevisiae* have been used to investigate the amino acid sequence requirements for these various post-transcriptional processes and for the interaction with redox partners, both *in vitro* and *in vivo*. Also other mutations affecting these processes have been investigated. Methionine excision is primarily dependent on the radius of gyration of the penultimate residue, but is partially influenced by the third residue (Tsunasawa *et al.*, unpublished). Various types of N-terminal sequences are acetylated (Moerschell *et al.*, unpublished), but the acetyltransferase coded by the *NAT1* and *ARD1* genes (Mullen *et al.*, unpublished) acts only on a subset of them. Heme lyase, encoded by *CYC3*, catalyzes heme attachment, which is required for import (1). In addition, certain amino acid residues of iso-1-cytochrome *c* (2) and the *CYC2* gene product (Schlichter *et al.*, unpublished) are also required for import. Apo-iso-1-cytochrome *c*, but not apo-iso-2-cytochrome *c*, is rapidly degraded if it is not imported (Dumont *et al.*, unpublished)

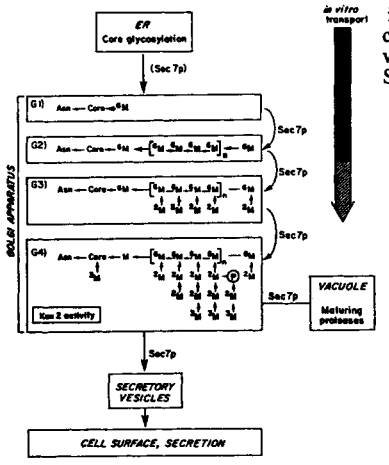


(1) Dumont *et al.*, EMBO J. **6**, 235-241 (1987).
(2) Dumont *et al.*, J. Biol. Chem. **263**, 15928-15937 (1988).

Post-Translational Control Mechanisms

N 019 FUNCTIONAL COMPARTMENTS OF THE YEAST GOLGI APPARATUS ARE DEFINED BY THE *sec7* MUTATION , Alex Franzusoff and Randy Schekman , Department of Biochemistry, Barker Hall, University of California, Berkeley, CA 94720.

Yeast cells bearing the temperature-sensitive *sec7* mutation accumulate exaggerated Golgi complexes and highly glycosylated proteins of the secretory pathway at the non-permissive temperature. The *SEC7* gene encodes a protein of 2008 amino acid residues (227 kDa). Polyclonal antisera, generated in response to *lacZ-SEC7* hybrid-fusion proteins, detect a 230 kDa polypeptide present in wild type yeast cell lysates. Cell fractionation experiments show that Sec7 protein (Sec7p) is present in both soluble and particulate fractions. The nature of Sec7p association with particulate structures was examined by treatment of cell lysates with reagents known to alter the distribution of sedimentable proteins. Sec7p is found in a detergent-insoluble complex, yet can be solubilized by low concentrations of urea in higher pH buffers. Hence, Sec7p is proposed to function in the yeast secretory pathway as a cytosolic protein that associates with higher order structures. This view is supported by results from indirect immune fluorescence of Sec7p in formaldehyde-fixed yeast.



To define more precisely the steps in the secretory pathway at which Sec7p functions, we have examined N-linked oligosaccharide processing on proteins accumulated in *sec7* yeast at the non-permissive temperature. Using specific immunological and enzymatic probes, we observe that *sec7* cells accumulate glycoprotein intermediates that lack distinct features of outer chain carbohydrate. Reconstitution of protein transport from ER to Golgi *in vitro* provides further evidence that enzymes responsible for the modifications associated with transit though the yeast Golgi apparatus do not reside in a single compartment. A model that accounts for the proposed compartmentation of processing within the yeast Golgi complex is shown in the figure.

N 020 MATURATION PATHWAYS OF PRECURSORS TO VACUOLAR HYDROLASES, Elizabeth W. Jones, Charles M. Moehle, Colleen K. Dixon and Vicki L. Nebes, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213

Several of the hydrolases found in the lysosome-like vacuole of the yeast Saccharomyces cerevisiae are synthesized as inactive precursors that must be proteolytically processed to yield mature enzymes. The DNA-derived amino acid sequence of PRB1, the structural gene of the subtilisin-like serine protease, protease B, is a 635 codon open reading frame corresponding to a polypeptide of nearly 70,000 Da(1). The mature N-terminus is amino acid 281; homology to the subtilisins ends at amino acid 575 of the ORF. Kinetic and mutant analyses indicate that at least three proteolytic cleavages are required to generate the active, mature enzyme. The first cleavage takes place in the endoplasmic reticulum, yields a 39kd product, and generates the mature N-terminus. We have yet to identify a mutant in which this cleavage fails to occur. We have tested all pep mutants, all prb mutants, the sec11 mutant, and the kex1 and kex2 mutants. Two proteolytic cleavages are required to generate the mature C-terminus. The first cleavage is dependent upon protease A function, for it doesn't occur in pep4 mutants. Site directed mutagenesis indicates that this cleavage will not occur if the N-linked glycosyl side chain, attached to the asparagine 41 residues from the C-terminus of the 40kd intermediate, is absent. The final cleavage converts the 37kd intermediate to the mature 31kd enzyme and fails to occur if the active site serine of the enzyme is replaced by an alanine, implying that protease B is required for its own synthesis. The prb1-628 mutation results in a retarded rate of conversion of the 37kd to the 31kd species. Supported by NIH AM18090 and GM29713.

1. Moehle, C.M., et al. (1987) Mol. Cell. Biol. 7:4390-4399.

N 021 PROTEIN SORTING TO THE YEAST VACUOLE

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The yeast vacuole is a lysosome-like organelle that contains a variety of hydrolases. We have employed a gene fusion approach to map the sequence determinants that direct accurate intracellular sorting of three vacuolar glycoproteins; two soluble hydrolases, carboxypeptidase Y (CPY) and proteinase A (PrA), and an integral membrane protein, repressible alkaline phosphatase (ALP). Different sized amino-terminal segments of CPY, PrA and ALP have been fused to mature, enzymatically active invertase [1,2]. We have found that short amino-terminal domains of preproCPY, preproPrA and proALP (50, 76 and 52 amino acids, respectively) contain sufficient information to 1) direct invertase entry into the secretory pathway and 2) quantitatively divert this normally secreted enzyme to the yeast vacuole. Mutational alterations of these vacuolar sorting signals lead to missorting and secretion of the otherwise wild type vacuolar proteins.

Efficient sorting of CPY-Inv hybrid proteins to the yeast vacuole by strains having no other source of active invertase results in the inability of such strains to grow on sucrose as their sole fermentable carbon source. We have exploited this Suc^- phenotype to select mutants that mislocalize the hybrid proteins to the cell surface. By this approach, we have identified more than 30 genes, the products of which appear to be required for accurate and efficient protein sorting to the vacuole [3,4]. These vacuolar protein sorting (vps) mutants missort and/or secrete several soluble vacuolar protein precursors including CPY, PrA and proteinase B. Most of these mutants have a greatly reduced affect on the sorting of ALP. General protein secretion appears to be unaffected in these mutants. We have been able to group the various vps mutants into three classes based on morphological differences. At present, we have cloned 5 genes which complement the vps mutant phenotypes. Interestingly, the DNA sequence of one of these genes has shown homology to a family of serine/threonine protein kinases. The vps mutants, in addition to providing access to gene functions that participate in the vacuolar protein sorting reaction, also may provide insights into the normal physiological role of the vacuole in yeast cell growth and development. Clearly, protein sorting to the vacuole, like other events in the secretory pathway, must be viewed as a complex process requiring the coordinate expression of many gene products.

1. Johnson, L.M., Bankaitis, V.A., and Emr, S.D. 1987. *Cell* 48:875-885.
2. Klionsky, D.J., Banta, L.M., and Emr, S.D. 1988. *Mol. Cell. Biol.* 8:2105-2116.
3. Banta, L.M., Robinson, J.S., Klionsky, D.J., and Emr, S.D. 1988. *J. Cell. Biol.* 107:1369-1383.
4. Robinson, J. S., Klionsky, D.J., Banta, L.M., and Emr, S.D. 1988. *Mol. Cell. Biol.* 8:4936-4948.

N 022 KAR2, THE YEAST HOMOLOGUE OF MAMMALIAN BiP/GRP78, IS REQUIRED FOR TRANSLOCATION OF PROTEINS INTO THE ENDOPLASMIC RETICULUM

Mark D. Rose, Joseph P. Vogel, Marci A. Scidmore and Leanne Misra, Dept. of Biology, Princeton University, Princeton, NJ, 08544-1014

In *Saccharomyces cerevisiae* the nuclear envelope remains intact throughout mitosis, meiosis and conjugation. Therefore formation of diploid nuclei during conjugation requires nuclear fusion (karyogamy). Karyogamy has been shown to be dependent upon three KAR genes, CDC4, activation by yeast mating hormone, and intact microtubules.

Molecular cloning and sequence analysis revealed that the KAR2 gene product is 67% identical to the mammalian BiP/GRP78 protein. The protein sequence is 63% identical to the yeast HSP70 gene, SSA1. KAR2 is not one of the previously identified yeast HSP70 genes. Several structural criteria indicate that KAR2 is the homologue of BiP/GRP78. First, KAR2 has a functional signal sequence for import into the endoplasmic reticulum (ER). Second, the gene lacks all the potential N-linked glycosylation signals present in highly conserved regions of HSP70 genes. Third, the protein terminates with the putative yeast "ER-retention" sequence, HDEL. Moreover, KAR2 is regulated in a fashion similar to the mammalian gene. Transcript levels are increased by glycosylation inhibitors (tunicamycin and 2-deoxyglucose) and by several sec mutations that cause accumulation of protein precursors in the ER.

KAR2 is essential for life as well as for karyogamy. A temperature sensitive mutation, kar2-159, causes arrest in the unbudded portion of the cell cycle after a shift to high temperature. The lethal defect of kar2-159 appears to be caused by a strong block in the secretory pathway. Secretory precursors of a number of proteins (invertase, alpha-factor, carboxypeptidase Y and KAR2) were found to accumulate rapidly. The precursors were found to retain their signal sequences and were not glycosylated. The precursor of at least one protein remains exposed to the cytoplasm, as revealed by protease protection experiments. These data indicate that BiP/GRP78 is required for the translocation of proteins into the lumen of the ER.

Isolation of suppressors of mutations in KAR2 has revealed one major gene (SKD1) that may functionally interact with KAR2. In addition, mutations in DPR1/RAM, thought to be involved in processing of RAS and a-factor, suppress and are suppressed by mutations in KAR2. The suppressor analysis should allow us to further elucidate the in vivo roles of KAR2 as well as the possible relationship between karyogamy and secretion.

Mechanisms of Pathogenesis

N 023 GENES FOR PHYTOALEXIN DETOXIFICATION ON SMALL, DISPENSIBLE CHROMOSOMES IN THE PLANT PATHOGEN *NECTRIA HAEMATOCOCCA*, Hans VanEtten, Vivian Miao and Alan Maloney, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Pathogenicity of the ascomycete *Nectria haematococca* on pea depends in part on the ability of the fungus to detoxify the pea phytoalexin pisatin. This reaction is catalyzed by pisatin demethylase, a substrate-inducible cytochrome P-450. The fungus possesses at least six genes for pisatin demethylase (*Pda* genes), any one of which is sufficient to confer pisatin demethylating ability; on pea seedlings however, high virulence is only associated with those genes conferring high levels of pisatin demethylating ability. Restriction and hybridization analyses using a cloned *Pda* gene suggests that there are two structural families of *Pda* genes, coincident with the different levels of virulence attributed to the activity levels of the Pda isozymes. These data suggest that fungal isolates carrying genes for efficient pisatin demethylating ability may have a selective advantage on peas over isolates which carry less effective *Pda* genes.

The *Pda* genes of *N. haematococca* at times show abnormal meiotic behavior, most often involving loss of the genes, as detected by pisatin demethylation, by hybridization with the cloned gene, or both. Although wild isolates of this pathogen have different electrophoretic karyotypes, most *Pda* genes map by hybridization to chromosomal DNA bands of less than 2 megabases resolved by pulsed-field gel electrophoresis. The meiotically unstable, anomalous inheritance of the *Pda6*-containing band suggests a mechanism by which *Pda* genes are deleted from the genome. Such small, dispensible chromosomes are similar in several respects to B chromosomes, but unlike conventional B chromosomes, the documented role of pisatin demethylation as a virulence factor in disease on pea suggests that the small *N. haematococca* chromosomes may contain genes conferring a phenotypic advantage to the fungus. Selective value of such genes as *Pda* in some contexts, e.g. on host plants, might maintain these chromosomes in the genome and increase diversity in the pathogen population.

N 024 HOST-PARASITE INTERACTIONS OF OPPORTUNISTIC FUNGI, Alayn R. Waldorf, Department of Biological Sciences, California State University, Hayward, Hayward, CA 94542

Prevention of aspergillosis and mucormycosis requires control of either spore germination and/or hyphal growth by the host. The host provides an important barrier to infection by control of spore or conidia germination, the critical step involving conversion of the fungus to its tissue-invasive form. Pulmonary defense against Aspergillus is dependent upon early killing of fungal conidia by alveolar macrophages. In contrast, prevention of mucormycosis appears to require inhibition of fungal spore germination by the alveolar macrophage. Since mucormycosis is most often seen in a setting of uncontrolled diabetes mellitus, and normal, but not diabetic alveolar macrophages provide an efficient defense against Rhizopus spores by inhibiting germination of spores in vivo and in vitro, phagocytosis-associated respiratory burst and lysosomal enzymes were studied in alveolar macrophages from normal and diabetic mice. Diabetic alveolar macrophages yielded higher respiratory burst activity than normal alveolar macrophages when exposed to opsonized zymosan or Rhizopus spores, as measured by luminol enhanced chemiluminescence. Yet, there was no significant difference in the mean rate of superoxide production by normal and diabetic alveolar macrophages. In contrast, diabetic alveolar macrophages had a decreased production of hydrogen peroxide in response to either soluble stimuli or Rhizopus spores, compared to normal cells. In assays of lysosomal enzyme activity, normal alveolar macrophages had significantly more lysozyme and acid phosphatase activity than diabetic macrophages. The normal and diabetic alveolar macrophage populations contain cells in different stages of maturation, and deficiencies in macrophages occur during streptozotocin-induced diabetes which correlates with the susceptibility of diabetics to mucormycosis.

The tissue invasive, hyphal form of the fungi which cause aspergillosis and mucormycosis are too large to be ingested by phagocytic cells. Although macrophages and monocytes can damage hyphae, the bulk of this role appears to fall upon the neutrophil. However, antihyphal mechanisms of neutrophils are not identical for all types of hyphae. Moreover, interactions of several potential oxidative and non-oxidative antihyphal mechanisms define the hosts ability to limit fungal infections.

Late Addition

N 025 GENETIC AND PHYSICAL ANALYSIS OF MEIOTIC CHROMOSOME SYNAPSIS AND RECOMBINATION IN *S. CEREVISIAE*. N. Kleckner, E. Alani, L. Cao and R. Padmore. Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA. 02138. (1) We have identified a meiosis-specific DNA "signal" which manifests itself in extracted chromosomal DNA as a double strand break (or pair of ends) at a specific site near *LEU2*. Appearance of this signal requires two genes needed for chromosome synapsis, *RAD50* and *SPO11*. Current results suggest that the signal appears at the time of homologue alignment, i.e. after DNA replication but before formation of synaptonemal complexes (SCs). It persists through most or all of the period when SCs are present, and then disappears, concomitant with the appearance of mature recombinant products. We suggest that this signal is a "feeler" used by one pair of sister chromatids to search out and interact with its homologous pair. (2) From temporal analysis of synchronous meiotic cultures, we conclude that both gene conversion and reciprocal exchange products (assayed by blot) occur either after or towards the end of the time when SCs are present; experiments to distinguish these possibilities are in progress. (3) The *RAD50* gene is required for SC formation and recombination in meiosis, and for DNA repair in vegetative growth. The properties of *rad50* mutants are accounted for by the general hypothesis that *RAD50* is required for homologous chromosomes to find one another. We have shown that, in meiosis, (i) *rad50* mutants are "asynaptic": axial cores form between sister chromatids, but mature SC does not and (ii) rarely, even *rad50* deletion mutants exhibit short stretches of SC. These observations suggest that *RAD50* is involved in homologue recognition and/or alignment rather than SC assembly *per se*. We have isolated a *RAD50* mutation that confers a meiotic defect but no defect in DNA repair; it maps in an ATP binding domain. We are investigating the possibility that *RAD50* is required both early in meiosis, for homologue alignment, and later, during recombinant formation, where its role would be like that in DNA repair.

Chromosome Structure and Function

N 100 OVER-EXPRESSION AND PURIFICATION OF YEAST TUBULINS IN SACCHAROMYCES CEREVISIAE. Susan L. Abrahamson, Brian Cheetham, John Carbon, Leslie Wilson. Department of Biological Sciences, University of California, Santa Barbara CA. 93117

Isolation and purification of assembly-competent tubulins from S. cerevisiae has always been problematic. We have developed an over-expression system in yeast utilizing both TUB1, encoding alpha-tubulin, and TUB2, encoding beta-tubulin. TUB2 was cloned downstream of the GAL1 promoter transcriptional activation site. A similar plasmid, obtained from Dr. Dan Burke, containing the essential alpha-tubulin gene TUB1 under similar GAL1 control was also used, allowing dual expression in yeast cotransformants. The two plasmids confer strong TUB1 and TUB2 over-expression when the cotransformants are induced on galactose. Over-expression was measured by Northern and Western analysis. Time course over-expression experiments indicate that the tubulins are continuously expressed over a thirty hour period post-galactose induction, although cell viability is observed to decrease. Tubulin proteins were partially purified by passage of crude cell lysates over a lactoperoxidase affinity column. The partially purified tubulin was able to specifically bind radiolabeled GTP and form filamentous structures in vitro, in reassembly experiments monitored by negative stain electron microscopy. Polymerization parameters and general biochemical characteristics of the over-expressed tubulins are currently under study.(Supp. by USPHS NS13560 & UC Biotech Grant)

N 101 EXPLORING THE RELATIONSHIP BETWEEN CHROMATIN STRUCTURE AND TRANSCRIPTIONAL REGULATION OF YEAST HEAT SHOCK GENES, Christopher C. Adams and David S. Gross, Department of Biochemistry and Molecular Biology, LSU Medical Center, Shreveport, LA 71130. A primary interest in our lab is the role that chromatin structure plays in the transcriptional regulation of yeast heat shock genes. Our current work centers on the two members of the *HSP90* gene family, *HSP82* and *HSC82*. While the two genes encode the same product, they are regulated quite differently: *HSP82* is expressed at a low basal level and is significantly inducible (~14-fold), whereas *HSC82* is transcribed at a very high basal level and is only modestly inducible (~2-fold). Both promoters contain multiple sequence elements with homologies to the revised heat shock element consensus, NTTCNNGAAN, as well as other sequences with regulatory potential (TATA boxes, putative repressor sequences, etc.). Previous work with *HSP82* has indicated that despite its substantial inducibility, the chromatin architecture of the transcriptional unit as well as 5' and 3' flanking sequences is unaltered during heat induction. We have recently initiated a complementary mapping study of the *HSC82* locus. Strikingly, we find that in contrast to the inducible gene, the 5' flank of *HSC82* exhibits a remarkable sensitivity to heat shock. In the non-heat-shocked state, an array of sequence-positioned nucleosomes spans the region -500 to -3500 and is interrupted by a single, bipartite hypersensitive site centered at -1900. Following heat shock, the chromatin structure of this entire 3 kb domain becomes markedly disrupted. Our preliminary results thus suggest the presence of a second heat-inducible gene adjacent to *HSC82* and within the same chromatin domain. We are currently investigating this and related possibilities.

N 102 YEAST PHO11 GENE: CLONING, LOCATION, SEQUENCING AND UPSTREAM ACTIVATION SITE, Shi- Zhou Ao, Jiang-yi Chen, Yi Gong, Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031, China.

PHO11 gene is one of yeast acid phosphatase gene family and inducible by low concentration of inorganic phosphate. A 5 Kb EcoRI fragment containing complete PHO11 gene was isolated from a yeast gene library. This fragment was analysed with restriction mapping, Southern hybridization and sequencing. Using pulsed field gradient gel electrophoresis technique, the PHO11 gene is located on chromosome VIII. The PHO11 gene codes for 467 amino acids as deduced from 1404 nucleotide sequences. The coding region and the 5'-flanking sequence of PHO11 gene share high homology with another repressible PHO5 gene in the nucleotide (74%) and the amino acid (85%) sequences . To study the mechanism of the regulation, the PHO11 gene was fused in frame with LacZ and assayed by bata-galactosidase activity. The 5'-flanking sequence of PHO11 gene was deleted by nuclease Bal31. The expression level of various deletions shows two upstream activating sequence regions are located between -407 and -464, -273 and -323 upstream of the initiation codon and activate expression of gene when inorganic phosphate is deleted.

N 103 A NON INTEGRATIVE LINEAR TRANSFORMATION VECTOR IN THE FUNGUS PODOSPORA ANSERINA AND ITS USE TO ISOLATE CHROMOSOMAL ELEMENTS, Christian Barreau, Jean Paul Javerzat, Michel Perrot and Joel Bégueret, Laboratoire de Génétique, Université de Bordeaux II, Avenue des Facultées, 33405 Talence, FRANCE.

The ends of the extrachromosomal Tetrahymena rDNA have been added to a plasmid containing the cloned ura5 gene of Podospora anserina. This vector can be linearized such that the two ends are constituted by the telomeric sequences. Transformation of the auxotrophic ura5 strain with this linear molecule give rise to 70-80% of unstable transformants containing the vector maintained at a low copy number as a linear non integrated plamid. Tetrahymena rDNA ends prevent integration of the vector and behave as functional telomeres in P. anserina (Perrot et al.; Mol. Cell. Biol., 7: 1725-1730, 1987). This plasmid behaves as a minichromosome lacking a strong replication origin (ARS) to be efficiently replicated and a functional centromeric sequence (CEN) to be mitotically and meiotically stabilized. Cloning of such chromosomal elements from genomic DNA has been attempted using the linear vector. Potential ARS elements which improve transformation efficiency have been isolated but these sequences do not enhance the copy number of the linear plasmid in the transformed strains. We are now looking for CEN sequences in order to stabilize the extrachromosomal plasmid.

N 104 A YEAST TELOMERE BINDING ACTIVITY BINDS TO TWO RELATED POLY($C_{1-3}A$) SEQUENCES AND IS INDISTINGUISHABLE FROM RAP1, Judith Berman, Mark S. Longtine, Nancy Maxfield Wilson, and Marie E. Petracek, Department of Plant Biology, University of Minnesota, St. Paul, MN 55108. Telomere Binding Activity (TBA), an abundant protein from Saccharomyces cerevisiae, was identified by its ability to bind to telomeric poly($C_{1-3}A$) sequence motifs (1). We analyzed the binding specificity of TBA for a number of different primary poly($C_{1-3}A$) sequences to determine whether the activity binds to a unique structure assumed by the irregularly repeating telomeric sequences or whether the activity recognizes and binds to a subset of specific sequences found within the telomere repeat tracts. Deletion analysis and DNAase I protection assays demonstrate that TBA binds to two poly($C_{1-3}A$) sequences that differ by one nucleotide. The methylation of four specific guanine residues, located at identical relative positions within these two binding sequences, interferes with TBA binding to the substrates. A synthetic oligonucleotide containing one of these TBA binding sites can function as a substrate for in vitro TBA binding and can compete for TBA binding to the other type of poly($C_{1-3}A$) binding site. The TBA binding sites share homology with the binding sites reported for the RAP1/GRFI and TUF transcription factors (2-4). Our TBA extracts bind to a number of RAP1/GRFI sub-strates and include a protein indistinguishable from RAP1 in binding specificity, size, and antigenicity. The affinity of TBA for the two telomeric binding sites is higher than its affinity for any of the other binding substrates tested.

1. Berman, J., C.Y. Tachibana, and B.-K Tye. 1986. Proc. Natl. Acad. Sci. USA 83: 3713-3717.
2. Shore, D., and K.A. Nasmyth, (1987) Cell 51: 709-719.
3. Buchman, A.R., W.J. Kimmerly, J. Rine and R.D. Kornberg, (1988) Mol. Cell. Biol. 8: 210-225.
4. Huet, J., and A. Sentenac, (1987) Proc. Natl. Acad. Sci. USA 84: 3648-3652.

N 105 CHROMOSOME MAPPING ON BASIS OF REAL MITOTIC CROSSING-OVER FREQUENCIES IN ASPERGILLUS NIGER, Cees J.Bos, Alfons J.M. Debets, Klaas Swart. Department of Genetics Agricultural University Wageningen, Dreyenlaan 2, 6703 HA Wageningen, The Netherlands.
Gene mapping in imperfect fungi on basis of mitotic recombination is often hampered by the absence of suitable distal selection markers and by the clonal appearance of recombinants that arise as sectors in heterozygous diploids. We established a collection of mutants of Aspergillus niger and constructed master strains for the assignment of genes to linkage groups (Curr.Genet.14, in press). Methods were developed to determine the linear order of genes of a linkage group by analysis of the genotype of diploids homozygous for a certain distal auxotrophic marker. Segregants were derived from several small conidiospore suspensions obtained from single conidial heads in order to avoid clonal effects. Auxotrophic recombinants were isolated quantitatively after enrichment by treatment of germinating spores with lytic enzymes. When the auxotrophic markers were in transposition, the homozygous diploids were haploidized, and the genotype of the diploid recombinants could be deducted from the haploid segregants. This method was also used to obtain haploid strains with closely linked markers. Such strains were subsequently used for genetic analysis as heterozygous diploids with recessive auxotroph markers in cis-position facilitated the analyses. The genetic lenght of different chromsome arms, and also of different chromsomes, can be compared as they are on the same scale. The total mitotic crossing-over frequency found for linkage group III was 10^{-3} with proC and cysA as most distal loci.

N 106 AN ELECTROPHORETIC KARYOTYPE OF ASPERGILLUS NIDULANS, Howard Brody and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, CA 93106
An electrophoretic karyotype of Aspergillus nidulans has been obtained using contour-clamped homogenous electric field (CHEF) gel electrophoresis. Six chromosomal bands were separated, with two of the bands migrating as doublets. Using the three S.pombe chromosomes as size standards, the sizes of the chromosomes are estimated to be between 2 and 7 megabases. Four of the eight genetic linkage groups were assigned to chromosomal bands by hybridization of CHEF gels with various radiolabeled probes each specific to a particular linkage group. CHEF analysis of reciprocal translocation strains gave chromosomal assignments for the four remaining linkage groups. In order of decreasing size, the A. nidulans chromosomes are; VIII, VII, II, (I and V), III, VI, and IV.

N 107 RECIPROCAL RECOMBINATION, GENE CONVERSION AND CHROMOSOME LOSS PHENOYPE IN IN THE *cdc6-1* MUTANT OF *S. cerevisiae*, Carlo V. Bruschi and John N. McMillan, Department of Microbiology and Immunology, Biotechnology Program, East Carolina University School of Medicine, Greenville, NC 27858.
We have analyzed the recombinational phenotype of *cdc6-1* mutants with respect to the relative proportion of reciprocal exchange and single-site gene conversion versus chromosome loss events. This study has been conducted with a diploid strain constructed to be homozygous for *cdc6-1* and to have three markers in heterozygous condition on chromosome VII: *leu1* (leucine auxotrophy), $cyh2^R$ (resistance to cycloheximide), and *ade* (adenine auxotrophy). The strain is also homozygous *ade1* and *ade2* and therefore red. When the temperature-sensitive strain is shifted for limited periods of time from room temperature to 30^o , 32^o or the fully restrictive 36^o C and then plated onto cycloheximide medium, the relative proportion of events resulting in cycloheximide resistant colonies varies with the temperature. In particular, reciprocal exchange and gene conversion decrease, whereas chromosome loss increases substantially. Control experiments with a similar strain homozygous for the wild-type *CDC6* indicated that cell viability is increasingly affected by temperature in the mutant but not in the wild-type The overall frequency of recombination and chromosome loss events at 32^o C was enhanced almost two orders of magnitude in the homozygous *cdc6* mutant.

This work was supported by the NASA Life Sciences Division Grant No. NAG 2-365 to C.V.B.

N 108 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE CEN2 REGION IN SCHIZOSACCHAROMYCES POMBE, Louise Clarke, Mary Baum and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, CA 93106

A 100 kb genomic SalI restriction fragment containing the centromere region of S. pombe chromosome II has been cloned in an artificial chromosome vector in both budding and fission yeasts. When present intact in S. pombe on a circular plasmid carrying an S. pombe ars element and selectable marker, this region functions as a centromere and converts the plasmid into an artificial chromosome, a fourth genetic linkage group. The functional minichromosome is stable through mitotic cell divisions (7×10^{-4} loss events/cell division) in S. pombe, and the genetic marker on the minichromosome segregates properly and with high fidelity through both meiotic divisions. The cen2 region contains three classes of moderately repetitive, centromere-specific DNA sequences, designated B, K, and L, and up to 80 kb of the region, including the repeated sequences, is heterochromatic-like and untranscribed. A plasmid integration-excision strategy has been used to obtain structural information across the entire cen2 region. Repeats B, K, and L are organized into four tandem units of approximately 18 kb each. Two of these units are arranged into a large inverted repeat structure centered around a 2-4 kb non-homologous sequence.

N 109 CELLULASE GENES FROM *PHANEROCHAETE CHRYSOSPORIUM*, Sarah F. Covert and Dan Cullen, Department of Bacteriology, University of Wisconsin, Madison, WI 53706; and Institute for Microbial and Biochemical Technology, Forest Products Laboratory, Forest Service - USDA, 1 Gifford Pinchot Dr., Madison, WI 53705. We are interested in cloning, sequencing, and characterizing the major cellulase genes of *Phanerochaete chrysosporium* BKM-1767. Towards these goals we have cloned and partially sequenced two putative cellulase genes from *P. chrysosporium* by hybridization to the *Trichoderma reesei* cellobiohydrolase I (CBHI) gene. The position of an intron is conserved between the two clones, and their deduced amino acid identity over 165 residues is 56%. When compared to the *T. reesei* CBHI sequence, the intron position is not conserved. The *P. chrysosporium* intron, however, is near one end of a highly conserved region corresponding to residues 146 to 209 of the *T. reesei* protein. Within this region one of our clones is 88% identical to the *T. reesei* sequence, and the other 61% identical. Further sequencing as well as studies of expression and linkage relationships are in progress.

This material is based upon work supported under an NSF Graduate Fellowship.

N 110 TWO GENES INVOLVED IN PENICILLIN BIOSYNTHESIS ARE CLUSTERED IN A 5.1 Kb SalI DNA FRAGMENT IN THE GENOME OF Penicillium chrysogenum, Bruno Díez, Santiago Gutiérrez, Christina Esmahan and Emilio Alvarez, Area of Microbiology, University of León, 24071 León, Spain

Forty-four out of 46 recombinant phages isolated from an EMBL3 library of P. chrysogenum DNA which gave positive hybridization with probes corresponding to the amino acid carboxyl terminal region of the isopenicillin N synthase (ips) gene also gave a positive signal when hybridized with a probe corresponding to the amino terminal end of the acyl-CoA:6-APA acyltransferase gene (aat) gene. A 5.1 Kb SalI DNA fragment common to nine randomly chosen recombinant phages showed strong hybridization with both the ips and aat probes. Linkage of both genes was also observed in three plasmids isolated from an independently constructed library of P. chrysogenum DNA in the bifunctional (E. coli-P. chrysogenum) shuttle vector pDJB3. Both genes are expressed in the same orientation from different promoters as concluded from subcloning experiments. They have been sequenced. A P. chrysogenum mutant npe8 pyrG which is altered in the 6-APA-acyltransferase was complemented by transformation with DNA of either recombinant phages or pDJB3-derived plasmids that contained the ips-aat cluster. Similarly, an isopenicillin N synthase deficient mutant of Acremonium chrysogenum (strain N2) was complemented by transformation with plasmids carrying both the aat and ips genes. One N2 transformant studied in detail had regained high levels of cyclase activity which is not normally present in A. chrysogenum. Linkage of both genes is of great interest since β-lactam biosynthetic genes appear to have derived from Streptomyces, where clustering of antibiotic biosynthetic genes is a well-known phenomenon.

N 111 CLONING OF ARS SEQUENCES FROM THE YEAST YARROWIA LIPOLYTICA. **Ph.FOURNIER, B.KUDLA, J-M NICAUD, M.CHASLES, J-W XUAN, C.GAILLARDIN. Lab. Génétique INA-INRA, Centre de Biotechnologies Agro-Industrielles, 78850 Thiverval-Grignon, FRANCE.**

We have recently reported (Yeast, 1988, 4, S127) that we could isolate ars sequences for the dimorphic yeast Yarrowia lipolytica in a morphogenesis mutant unable to produce mycelium. Here we show that these sequences can directly be isolated in a filamentous strain, but at a lower frequency. They are mitotically more stable in this genetic background. The nucleotide sequence of 1305 nucleotides encompassing one of these ars fragments has been established and shown to display a complex pattern of repetitions, including stretches imperfectly matching to the S.cerevisiae ars consensus. The amplification achieved on the expression of several genes by ars plasmids has been measured and indicates a copy number comprised between 3 to 7. Presence of direct repeats in ars plasmids has been shown to cause a high rate of deletion by homologous recombination during the transformation process. The deletion frequency is correlated to the size of the repeat, and depends on the genetic background.

N 112 ISOLATION OF CONDITIONALLY LETHAL MUTANTS OF *SACCHAROMYCES CEREVISIAE* DEFECTIVE IN MITOTIC CHROMOSOME SEGREGATION, Arlene M. Gaudet, William E. Payne and Molly Fitzgerald-Hayes, Department of Biochemistry, University of Massachusetts, Amherst, MA 01003
We are attempting to identify and isolate genes encoding factors which are components of the kinetochore of *Saccharomyces cerevisiae*. Strains carrying a mutant centromere were mutagenized and colonies screened for increased nondisjunction/loss of the mutant chromosome using the *SUP11*-based colony color assay. This screen has yielded several mutants which show increased colony sectoring indicating increased chromosome nondisjunction or loss per cell division. Strains which also display a conditionally lethal phenotype which cosegregates with the increased sectoring phenotype were chosen for further study. These mutants show levels of nondisjunction one to several orders of magnitude over unmutagenized strains. The temperature-sensitive phenotype will be exploited to clone complementary DNA from a yeast genomic library.

N 113 IN VIVO FOOTPRINTING REVEALS UNEXPECTED FEATURES WITHIN THE PROMOTER OF A YEAST HEAT SHOCK GENE, David S. Gross, Kerry W. Collins, Karen E. English, and Seewoo Lee, Department of Biochemistry and Molecular Biology, LSU Medical Center, Shreveport, LA 71130. We report here the identification of regulatory protein contacts and characterization of DNA helical topology within the promoter region of the heat-shock inducible *HSP82* gene of *Saccharomyces cerevisiae*. We have employed two complementary strategies: [1] dimethyl sulfate (DMS) methylation of intact cells, followed by piperidine-induced cleavage of purified DNA, to probe tight protein-DNA interactions within the major groove; and [2] hydroxyl radical (·OH) cleavage of chromosomal DNA in spheroplast lysates, both to probe protein-DNA interactions at the sugar-phosphate backbone as well as to elucidate the *in vivo* helical periodicity of DNA. DMS methylation protection reveals the constitutive binding of a protein, presumably heat shock factor (HSF), to 7 guanines within the major groove of the principal heat shock element (HSE 1) located between -178 and -158. Similarly, hydroxyl radical detects the constitutive presence of a factor which protects the sugar-phosphate backbone within and flanking the TATA element positioned at -80; this protection spans 18 bp, beginning at -85 and extending to -73. Thus, despite being ~14-fold inducibile, the *HSP82* promoter is *constitutively* protected from chemical modification at both *cis*-acting elements. Hydroxyl radical cleavage reveals the presence of two other unusual features within the *HSP82* promoter region: [1] A ladder of 14 regularly spaced bands spanning from -142 to -286, exhibiting roughly 10-11 bp periodicity, and seen in both chromatin and naked genomic DNA; and [2] A string of 4 purines (5'-GAAG-3'), positioned between -56 and -59, and located within a purine-rich block on the lower strand, that are hypersensitive to ·OH in chromatin.

N 114 CLONING AND FUNCTIONAL ANALYSIS OF THE CEN1 REGION OF SCHIZOSACCHAROMYCES POMBE, Karen M. Hahnenberger, John Carbon and Louise Clarke, Department of Biological Sciences, University of California, Santa Barbara, CA 93106
A 65 kb genomic SalI restriction fragment containing the centromere region from chromosome I of S. pombe was cloned in S. cerevisiae using a yeast artificial chromosome vector. When transformed into S. pombe, this 78 kb linear minichromosome is mitotically stable ($5x10^{-3}$ loss events/cell division) and behaves as an independent linkage group which segregates properly through meiosis. A 40 kb circular minichromosome derived from the 78 kb linear minichromosome was found to exhibit partial centromere function. The 40 kb circular minichromosome is 3-fold less stable mitotically than the 78 kb linear and segregates 2+:2- through meiosis in 93% of the tetrads analyzed. However, in half of these tetrads the circular minichromosome undergoes precocious sister chromatid separation in the first meiotic division. Increasing the size of the 40 kb circular minichromosome to 75 kb by integration of additional vector sequences increases its mitotic stability over 100-fold but has no effect on its meiotic segregation pattern. This suggests that the 40kb minichromosome contains all of the sequences which are necessary for mitotic and meiotic stability but lacks sequences which are required to maintain sister chromatid attachment in the first meiotic division.

N 115 A FUNCTIONAL YEAST CENTROMERE IS ONLY 125 BP LONG, Johannes H. Hegemann, Philip Hieter [1], James H.Shero [1] and Guillaume Cottarel, Institut fuer Mikro- und Molekularbiologie, JL Universitaet, Frankfurter Str.107, 6300 Giessen, Federal Republic of Germany and [1] Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205. The S.cerevisiae centromere DNA contains a conserved region ranging from 111 to 119 bp in length and is characterized by the 3 conserved DNA elements CDEI, CDEII and CDEIII. We isolated a 125 bp CEN6 DNA fragment bearing only CDEI, II and III (ML CEN6) and tested it with the recently developed chromosome fragment assay and on plasmids. The results allow us to conclude that this DNA fragment is sufficient to fulfil complete mitotic centromere function. The meiotic activity of ML CEN6 is indistinguishable from that of a 1.16 kb CEN6 DNA fragment when placed on linear artifical chromosomes.

N 116 AN ELECTROPHORETIC KARYOTYPE FOR THE PATHOGENIC FUNGUS, *PHYTOPHTHORA MEGASPERMA*, Barbara J. Howlett, Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia

Chromosomal DNA has been prepared from mycelial spheroplasts of *Phytophthora megasperma* and resolved by a pulsed field gel electrophoresis system which uses contour-clamped homogeneous electric fields (Chu *et al.*, 1986). Nine chromosomal DNA bands were separated, the smallest being about 1.4 megabases in size; several larger DNAs were unresolved under all conditions tested. The estimated sizes were based on migration rates relative to those of chromosomal DNA of *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and a *Neurospora crassa* translocation strain which has a minichromosome.

Currently a genomic library of *Phytophthora* DNA is being screened with several fungal genes which hybridize to DNA on Southern blots. The homologous *Phytophthora* genes will be used to develop a map using blots of chromosomal DNA.

Chu, G., Vollrath, D. and Davis, R.W. (1986) Science 234, 1582-1585.

N 117 THE STRUCTURAL AUTONOMY OF A YEAST CENTROMERE. Margaret Kenna and Kerry Bloom. Dept. of Biology. University of North Carolina, Chapel Hill, N.C. 27599.

CEN3 DNA is organized in chromatin such that a 220-250 bp region encompassing the elements of centromere homolgy is resistant to nuclease digestion. This protected region is no longer apparent when isloated nuclei are treated with high concentrations of NaCl prior to nuclease digestions. Alternatively, the complex is disrupted in selective point mutations that inactivate CEN function. The chromatin components (CEN-binding proteins) involved in the structural integrity of this chromosomal domain are therefore required for function. We have recently examined torsional constraints on the centromere structure. A modified indirect end-labeling experiment was performed by first digesting yeast nuclei with a restriction enzyme that cuts close to the protected structure surrounding CEN3 in chromosome III, followed by digestion with micrococcal nuclease. The result shows that an incision in the chromosome adjacent to the centromere does not perturb the structural organization of the centromere. Further, the protected structure is still present on a CEN fragment that has been excised from the rest of the chromosome. This demonstrates that at least the set of proteins conferring the nuclease resistant structure is not dependent on the torsional state of the surrounding DNA. Additionally, no change in the centromere structure was evident in several topoisomerase mutants of yeast that were examined (top1, top 2, top1top2). We are currently using the topoisomerase mutants to determine the mechanism of transcriptional inactivation of this cis-acting sequence.

N 118 PURIFICATION AND CHARACTERISATION OF PROTEINS THAT BIND TO CDE III OF S.cerevisiae CENTROMERES, Johannes Lechner and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, CA 93106

Of the three S.cerevisiae centromere sequence domains (CDE I,II and III) CDE III is most important for proper centromere function. Proteins that form complexes with CDE I+II or CDE III can be detected in crude extracts by gel retardation assays. Complex III - in contrast to complex I+II - is not formed with a centromere DNA fragment containing a point mutation (CF mutation) that eliminates centromere function in vivo . Proteins participating in the formation of complex III are therefore likely to play an important role in chromosome segregation. CDE III binding proteins are currently purified about 100,000 fold by common procedures. The final affinity chromatography step is performed either with wild-type CEN DNA or DNA containing the CF mutation. Since CDE III binding proteins attach only to wild-type DNA, comparison of proteins eluting from the two matrixes can identify CDE III binding proteins after SDS PAGE. At least one of these proteins is phosphorylated and the phosphorylated state of this protein is a necessary prerequisite for the formation of complex III.

N 119 CHARACTERIZATION OF THE CIS-ACTING DETERMINANTS OF TELOMERE ADDITION AND STABILITY IN YEAST, Arthur J. Lustig, Program in Molecular Biology and Virology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, N.Y. 10021

The replication of eukaryotic chromosomal telomeres appears to proceed via a template independent process unlike conventional semi-conservative DNA synthesis. This process results in the variable addition of G-rich simple sequence tracts onto pre-existing tracts during DNA synthesis. In yeast, these tracts consist of poly ($C_{1-3}A$). The tract length varies in different wild type strains of yeast from ≈150-800bp, each strain maintaining lengths at a strain-specific equilibrium. It has previously been demonstrated by others that linearized plasmids terminating in either the Tetrahymena poly (C_4A_2) or Oxytricha poly (C_4A_4) telomeres are capable of being modified by the addition of yeast poly ($C_{1-3}A$) tracts, resulting in the propagation of such plasmids as linear molecules in vivo. This process is likely to reflect at least some of the mechanisms operating during normal telomere replication.

We have utilized the ability of yeast to "heal" such heterologous termini to examine the sequence requirements of the telomere addition reaction. To this end, we have constructed an extensive series of artificial telomeres of known sequence in vitro, linked these termini to a linearized replicating plasmid, and transformed this ligation mix into a rad52-containing strain in order to suppress mitotic gene conversion events. Using this system, we have determined that relatively long (≈40 bp) tracts of specific sequence are required for efficient addition and that the sequence similarity of artificial telomeres to endogenous yeast telomeres must be quite high in order for efficient addition to take place.

Several laboratories (1,2) have isolated a protein, encoded by the RAP1 gene, that binds to a specific variation of the poly ($C_{1-3}A$) tract, 5'-$ACAC_3(AC)_3C$-3'. We have tested the impact of disrupting the RAP1 binding sites, present in control poly ($C_{1-3}A$)-containing artificial telomeres, on telomere addition and stability in both $Rad52^+$ and $Rad52^-$ stains, using the assay described above. We have found that disruption of the RAP1 sites within the artificial telomeres have little impact on telomere addition in $Rad52^-$ strains. However, in $Rad52^+$ strains, RAP1 site disruption results in a major increase in the formation of plasmid recombinant products. On the basis of these results, we propose that one role of the RAP1 protein may be to suppress double-strand initiated recombination at the telomere.

1. Shore, D. and Nasmyth, K . (1987) Cell 51: 721-732.
2. Buchman, A.R., Kimmerly, W.J., Rine, J., and Kornberg, R.D. (1988) Mol.Cell. Biol. 8: 210-225.

N 120 A 35 Kb DNA FRAGMENT CARRYING TWO PENICILLIN BIOSYNTHETIC GENES IS AMPLIFIED IN HIGH PENICILLIN PRODUCING STRAINS OF P. CHRYSOGENUM, Jose L. barredo, Jesus M. Cantoral, Eduardo Montenegro and Juan F. Martín, Area of Microbiology, University of Leon, 24071 Leon, Spain

A 19.5 Kb fragment of P. chrysogenum AS-P-78 DNA was cloned in EMBL3 phage vector. The isopenicillin N synthase (ips) and acyl-CoA:6-APA acyltransferase (aat) genes of P. chrysogenum were located in the cloned DNA. The 19.5 Kb DNA fragment was mapped by double and triple digestions with several endonucleases and the ips and aat genes were located by hybridization with probes corresponding to internal fragments of each gene. A low penicillin producing strain (P. chrysogenum Wis 54-1255) and two high producing strains (AS-P-78 and P_2) showed hybridizing fragments of identical size in their chromosomes. By dot-blot hybridization of serial dilutions of total DNA of the three strains it was shown that the intensity of all hybridizing bands was much higher in strain AS-P-78 and P_2 than in Wis 54-1255. Hybridization with probes corresponding to fragments which mapped upstream or downstream of the ips-aat region revealed that a fragment of at least 35 Kb DNA has been amplified in the high penicillin producing strains. The amplified region did not include the pyrG gene which encodes OMP-decarboxylase, an enzyme involved in pyrimidine biosynthesis, which is used as a selective marker in fungal cloning vectors.

N 121 CHARACTERIZATION OF THE CORE HISTONE GENES OF ASPERGILLUS NIDULANS, Gregory S. May, Steve Denison and Ann Ehinger, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030

We are characterizing the core histone gene family of Aspergillus nidulans. All the core histone genes are unique except the histone H4 gene. There appear to be two H4 genes, one expressed and the other apparently silent. As previously reported for the H2A gene (G.S. May and N.R. Morris, 1987, Gene 58, 59-66) introns interrupt the H2B, H3 genes and the expressed H4 gene. In contrast to the H2A gene which has three introns, the H2B, H3 and H4 genes each has two introns. The introns of the H2B gene split codons for amino acids 58 and 111, those of the H3 gene split codons for amino acids 9 and 45, and the introns of the H4 gene split codons for amino acids 4 and 96. The histone H3 and H4 genes are separated by approximately 700 base pairs and are divergently transcribed. The H2A and H2B genes are similarly arranged and are also transcribed divergently. Each of the core histone gene pairs has been mapped to linkage group by hybridization to chromosome sized DNA separated by CHEF gel electrophoresis. The H3-H4 pair is located on linkage group VIII, while the H2A-H2B pair hybridized to a pair of bands containing linkage group III and an as yet unidentified linkage group. Current efforts are directed toward the isolation of the silent H4 gene to characterize it fully and the precise assignment of the H2A-H2B pair to linkage group.

N 122 KARYOTYPIC VARIATION AMONG FOURTEEN RACES OF USTILAGO HORDEI ANALYZED BY CHEF ELECTROPHORESIS, Kevin McCluskey and Dallice Mills, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902

Ustilago hordei (Pers.) Lagerh., a basidiomycetous fungus, is the causal agent of covered smut of barley. The karyotypes of fourteen races of U. hordei were analyzed with CHEF-II electrophoresis. The number of chromosome-sized DNAs varied from twelve to sixteen and ranged in size from 235 kilobases to approximately 2.5 megabases. Although no two races were found to have the same complement of chromosome-sized DNAs, some bands were present in every race, including the 235 kilobase band and a high molecular weight band. An analysis of the karyotypes of the four meiotic products arising from a single teliospore was carried out for several races. Typically the karyotypes of the products of a single meiotic event were conserved. Variation in nuclear number in secondary sporidia was assessed with the fluorescent dye DAPI. The relevance of the karyotypic variation to the concept of race is discussed in the context of the inbreeding nature of smut fungi.

N 123 NON-TRANSMITTANCE THROUGH MEIOSIS OF A GENE FOR PISATIN DEMETHYLASE IN *NECTRIA HAEMATOCOCCA* MP VI IS CORRELATED WITH A CHANGE IN ELECTROPHORETIC KARYOTYPE, V. Miao and H.D. VanEtten, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The phytoalexin pisatin is produced by garden pea (*Pisum sativum*) in response to challenge by the pea pathogen *Nectria haematococca* MP VI. *Nectria* strains having any member of a family of *PDA* (pisatin demethylating ability) genes encoding pisatin specific cytochrome P-450 enzymes have a pisatin detoxifying phenotype (Pda+). Tetrad analysis of conventional crosses between strains with *Pda6* genes, or between *Pda6* containing (Pda+) and non-detoxifying (Pda-) strains, sometimes resulted in an excess of Pda- progeny; loss of the Pda+ phenotype was correlated with loss of a 9-10kb restriction fragment of genomic DNA which cross-hybridizes with a cloned *PDA* gene (*PDAT9*). Recently we found that the *PDAT9* probe hybridized to an approximately 1.8 Mb band of chromosomal DNA in partial karyotypes resolved by pulsed field electrophoresis (CHEF); strains which had lost *Pda6* did not have this band. In some instances, a novel 1 Mb band appeared in the Pda- isolates. We suggest that extra Pda- progeny may be generated by large deletions which alter the electrophoretic karyotype.

N 124 GROWTH OF HAPLOID STRAINS OF TILLETIA CONTROVERSA AND T. CARIES IN SUSCEPTIBLE AND RESISTANT CULTIVARS OF WHEAT, AND GENETIC ANALYSIS OF AN INTERSPECIFIC CROSS [1]Dallice Mills, [2]Frances Trail and [1]Brian Russell, [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, and [2]Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva NY 14456
Tilletia caries, (DC) Tul. and *T. controversa* (Kuhn) are the causal agents of common smut and dwarf smut of wheat, respectively. Infection hyphae of each fungus are produced by the fusion of sexually compatible basidiospores, and are thought to penetrate the coleoptile of germinating seeds and become established in the shoot meristem. Subsequently, teliospores are produced in smut balls that replace the wheat kernel. To develop techniques for making intra- and inter-species crosses of these fungi *in planta*, wild type and mutant haploid strains of each fungus were injected by hyperdermic needle into the uppermost node of flagleaf-stage plants, and analyzed for their ability to grow in susceptible and resistant cultivars of wheat. Mutant and wild type strains were recovered from both susceptible and resistant wheat plants 3 to 6 weeks after inoculation. Haploid strains that appeared to be compatible in interspecific mating tests were then crossed *in planta* and in wheat organ culture, and teliospores resulting from one interspecific cross were analyzed. The optimum temperature for teliospore germination was controlled by a dominant gene(s) from the *T. controversa* parent, whereas the onset of germination was intermediate relative to these two fungi. A spontaneous mutation to cycloheximide resistance in the *T. controversa* parent assorted independently from mating type alleles in 101 progeny from this cross, consistent with Mendelian assortment of unlinked genes.

N 125 USE OF SYNTHETIC *CEN3* DNA TO STUDY CENTROMERE FUNCTION IN THE YEAST *SACCHAROMYCES CEREVISIAE*, Mike Murphy and Molly Fitzgerald-Hayes, Biochemistry Department, University of Massachusetts, Amherst, MA 01003; Dana Fowlkes, Department of Pathology, University of North Carolina, Chapel Hill, NC 27514

Centromere DNA from the budding yeast *Saccharomyces cerevisiae* contains three conserved DNA elements, CDE I, II, and III. CDE I is an 8 bp sequence at one end of the centromere. CDE III is a 25 bp sequence containing partial dyad symmetry located at the other end of the centromere. The central region, CDE II, is 78 to 86 bp in length, greater than 90% A+T-rich, and contains runs of A and T residues.

Conventional mutagenesis techniques have revealed much about sequences necessary for meiotic and mitotic centromere function; however, some mutations cannot be made using these techniques. Therefore, we made several centromeres by annealing synthetic oligonucleotides designed to address important questions about centromere structure and function. Preliminary results indicate that the conserved centromere DNA elements alone are sufficient for wild-type centromere function. Currently, we are investigating the effect of bent DNA in CDE II. Finally, preliminary results indicate that inversion of CDE III abolishes centromere function whereas inversion of CDE I does not.

N 126 IDENTIFICATION OF MUTATIONS IN GENES ENCODING FACTORS POTENTIALLY INVOLVED IN KINETOCHORE STRUCTURE-FUNCTION IN S. cerevisiae, Francois Perier and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.
Using a genetic approach, we are trying to identify genes whose products bind centromere-specific DNA sequences in S. cerevisiae. A screen involving the detection of mutants deficient in recognition of a 61-bp CEN11 fragment containing the 25-bp CDE III region and flanking sequences was developed. The target DNA fragment was inserted between the GAL1,10 UAS and the TATA box of a GAL1-lacZ fusion in a yeast plasmid. Cells containing the vector without insert form dark blue colonies on galactose/X-gal plates while those harboring the vector plus insert are pale blue on the same medium. The latter were mutagenized and dark blue clones were further characterized. Five of such "blu" mutants were identified. These mutations are recessive and appear to belong to three different complementation groups. Preliminary results indicate that mutants belonging to only one of these classes undergo significant levels of chromosome non-disjunction.

N 127 THE MOLECULAR ARCHITECTURE OF YEAST CENTROMERES, Michael J.S. Saunders[1], Michael Grunstein[2], and Kerry Bloom[1], [1]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, and [2]Molecular Biology Institute, UCLA, Los Angeles, CA 90024

The centromeric DNA of Saccharomyces cerevisiae is packaged in vivo into a strongly nuclease resistant particle that encompasses approximately 200bp of CEN DNA. The presence of this nuclease resistant structure has been correlated with centromeric function, and the protection is presumed to be afforded by centromere specific DNA-binding proteins. We are studying the molecular architecture of the centromere complex and its protein components in order to elucidate the mechanisms of microtubule attachment and chromosome movement. We have demonstrated the probable involvement of nucleosomes in forming the centromeric complex by investigating the nuclease sensitivity of centromeric chromatin in yeast strains in which the expression of histone H2B or H4 can be regulated. Transcriptional repression of histone H2B or H4 leads to increased nuclease sensitivity of CEN3 chromatin. We have developed a technique to affinity purify centromere complexes from yeast chromatin. A DNA molecular cassette, flanked by EcoRI sites has been constructed that contains a 289bp CEN3 fragment placed adjacent to a synthetic symmetrical lac operator site. This cassette can be cleaved out of yeast chromatin and incubated with lac repressor-ß-galactosidase fusion protein. Anti ß-gal sepharose is then used to affinity purify the centromeric cassette. We are currently using this technique in an attempt to identify centromere specific protein components.

N 128 α-GALACTOSIDASE GENE FROM SACCHAROMYCES CEREVISIAE VAR.CARLSBERGENSIS: MEL2, Hilkka Turakainen *, Sirpa Aho ** and Matti Korhola **, *Department of Genetics, University of Helsinki, SF-00100 Helsinki ; **Research Laboratories of the Finnish State Alcohol Company (Alko Ltd), SF-00101 Helsinki, Finland.

Only some strains of S.cerevisiae carry a MEL gene and, therefore, produce α-galactosidase. The MEL1 gene was earlier cloned from S.cer.var.uvarum (1) and sequenced (2). Cloned MEL1 gene was used as a probe in hybridization experiments with a genomic DNA digest of S.cer.var.carlsbergensis (NCYC 396). The probe hybridized to a single 10 kb EcoRI fragment. This fragment was extracted from the agarose gel and ligated to an EcoRI-digested dephosphorylated plasmid pBR322. We determined the nucleotide sequence of a 3.6 kb region containing the MEL2 gene. The amino acid sequence deduced from the nucleotide sequence of the MEL2 gene showed 84% homology with the MEL1 gene product. The size of the in vivo mRNA was estimated by northern hybridization analysis to be about 1.5 kb. This is in good agreement with the size of the presumed protein (52 kd). The 10 kb EcoRI fragment and a 5.5 kb BglII fragment conferred the ability to produce α-galactosidase when introduced via a shuttle vector into a Mel^- yeast strain. The expression of MEL1 and MEL2 genes was studied in the donor yeast cells and in S.cer.P18-1a cells transformed with the shuttle vector containing the MEL1 or MEL2 gene.

References: 1. Ruohola et al.(1986) FEMS Microbiol.Lett.34:179-185;
2. Liljeström (1985) Nucleic Acids Res.13:7257-7268.

N 129 A NOVEL TYPE I TOPOISOMERASE FROM YEAST MITOCHONDRIA, R. Uthayashanker and H. Peter Zassenhaus, Department of Microbiology, St. Louis University School of Medicine, St. Louis, Mo., 63104. Type I topoisomerases are a class of enzymes that reduce the linking number of covalently closed DNA in steps of one by transiently introducing single strand breaks in the DNA. These enzymes have been proposed to have a function during transcription, to relieve the torsional stress associated with the RNA polymerase, and to be involved in DNA recombination whether at homologous or site-specific regions. We will report on the characterization of a novel ADP-dependent Type I topoisomerase detected in the mitochondria of Saccharomyces cerevisiae. This enzyme is distinct from the previously described nuclear-located yeast topoisomerases. We will also report on a mutant which may identify the gene encoding the mitochondrial topoisomerase.

N 130 THE OBF1 BINDING SITES IN ARS 121 ARE IMPORTANT FOR PLASMID MAINTENANCE, Scott S. Walker, Stephen C. Francesconi, and Shlomo Eisenberg, Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06032. Saccharomyces cerevisiae provides an amenable model system to study the genetics and biochemistry of the initiation of DNA replication in eukaryotes. Yeast DNA replication is initiated at multiple site along each chromosome (replicons). Several of these replicons appear to be initiated within or near specific DNA sequences (ARS) shown to provide origin function to plasmids. By analogy to other replication systems, we presume that protein factors are involved activation and regulation of yeast origins of DNA replication. We have identified and purified a protein, OBF1 (127 kd), that binds to several cloned ARS's (ARS's 120, 121, 131C, HMR E, 1). To better study the relationship between OBF1 binding and ARS function we have subcloned ARS 121 to a 480 bp fragment. This DNA fragment contains a high as well as a low affinity OBF1 binding site and ARS activity. Using a DNA mobility retardation assay we determined the effects, on protein binding, of mutagenizing either or both of the binding sites in ARS 121. Substitution mutants within either of the two sites obliterate detectable in vitro protein binding to that site. Linker substitution (6bp) mutations within the high affinity site show effects identical to those of the complete substitution mutant, whereas substitutions outside the binding site bind OBF1 normally. We also tested the mitotic stability of centromeric plasmids bearing wild type and mutagenized copies of ARS 121. Eighteen base pair substitutions within either of the two OBF1 binding sites reduces the percent of plasmid containing cells in the population from 88% to 63% under selective growth and from 46% to 20% under nonselective growth (for 10-12 generations). Furthermore, linker substitutions within the major binding site show similar defficiencies in plasmid stability. In contrast, plasmids containing linker substitutions in sequences adjacent to the high affinity site do not exhibit reduced stability. All these results, taken together, strongly support the notion that the OBF1 binding sites and the OBF1 protein are important for normal ARS function as an origin of replication.

Abstract Withdrawn

N 132 DELETION AND REGULATION OF α-AMYLASE GENES IN *ASPERGILLUS AWAMORI.* LORI J. WILSON, RANDY M. BERKA, AND MICK WARD. Genencor, Inc. South San Francisco, CA 94080.

Aspergillus awamori contains two very similar α-amylase genes. Vectors have been constructed to delete one or the other or both of these genes by replacing them with a selectable marker. These deletions are being used to test whether:

a) both genes are functional

b) there are additional α-amylase genes

c) the genes are regulated differently

d) the regulation is at the level of transcription or translation

N 133 PROPAGATION OF A CONDITIONAL CENTROMERE PLASMID IN *Saccharomyces cerevisiae* POPULATIONS, Dane Wittrup, Avantika Patel*, Barry Ratzkin*, and James Bailey, California Institute of Technology, Pasadena, CA 91125, *Amgen Inc., Thousand Oaks, CA 91320. A prototype yeast expression vector was constructed which contained the following sequences: the CEN3 centromere downstream of the CUP1 copper-inducible promoter; a gene encoding resistance to the antibiotic G418 constitutively expressed from the yeast PGK promoter; and a lacZ gene expressed from a GAL10-CYC1 hybrid promoter. Induction of CUP1 transcription interferes with the partitioning function of the centromere, causing copy number to be amplified in a subpopulation of cells. Growth of cells with amplified copy number is selected by increasing G418 concentration in the growth medium. β-galactosidase activity is used as a marker for copy number by assuming proportionality between gene dosage and lacZ expression. A flow cytometric assay of β-galactosidase activity at the single-cell level was used to measure the distribution of copy number in the population. The observed copy number distributions are consistent with a mathematical model which postulates that centromere-containing plasmids at copy numbers in excess of 6 are subject to biased segregation. This assumption is a simplification of the general hypothesis that some component of the mitotic apparatus is titrated by increasing the number of centromeres in the cell.

Reproduction and Cell Proliferation

N 200 DEVELOPMENT CAUSES NUTRIENT LIMITATION IN ASPERGILLUS NIDULANS. Thomas H. Adams and William E. Timberlake, Departments of Genetics and Plant Pathology, University of Georgia, Athens, GA 30602.

Microbial differentiation is typically induced by environmental stresses such as nutrient limitation. We have shown that forced expression of Aspergillus nidulans brlA or abaA, two genes that are required for asexual reproduction, induces development and inhibits vegetative growth under conditions that normally suppress development while allowing vegetative growth (Adams et al., 1988, Cell 54:353; Mirabito et al., In Preparation). Activation of development under these conditions leads to global changes in cellular metabolism resulting in large decreases in total RNA and protein levels. These reductions are at least partly due to an inhibition of new RNA and protein synthesis. The metabolic changes brought about by development also include a depletion of at least some enzymes needed for nutrient acquisition. Thus, inducing development leads to nutrient limitation, an inducer of development. We propose that developmentally-induced nutrient limitation reinforces a primary stimulus such that, once initiated, development proceeds regardless of environment.

N 201 GENETIC MANIPULATION OF POLYPLOID BREWER'S YEAST: CONSTRUCTION OF AN IMPROVED BREWING HYBRID OF Saccharomyces cerevisiae AND Saccharomyces uvarum (carlsbergensis), Carl A. Bilinski, Research Department, Labatt Brewing Company Limited, London, Ontario, Canada N6A 4M3 and Gregory P. Casey, Department of Applied Microbiology and FoodScience, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

Improved sporulation procedures applied to presumed weakly-sporogenic polyploid ale and lager production strains has permitted isolation of a- and α-maters for use in cross-breeding. A novel interspecies hybrid constructed by simple mating of ale and lager meiotic segregants was characterized and it was found possible to circumvent the problem of limited genetic markers by application of Transverse Alternating Pulsed Field Electrophoresis (TAFE) as a tool to trace chromosome inheritance. In comparison to its parents, the hybrid displayed in wort fermentation trials faster attenuation and ethanol production rates that were correlated with enhanced maltose uptake, an observation attributable to MAL gene dosage effects and/or positive heterosis. Beer produced with the species hybrid was deemed to be very palatable and lacked the sulphury character of lager but had the estery aroma of ale.

N 202 SACCHAROMYCES CEREVISIAE MUTANTS DEFECTIVE IN CHITIN SYNTHESIS,
C.E. Bulawa and P.W. Robbins, MIT, Cambridge, MA 02139.

Chitin is a major component of the division septum of S. cerevisiae. Two enzymes catalyze chitin synthesis in vitro; both are zymogens that are activated by trypsin. Chitin synthase I is not required for chitin synthesis in vivo; its cellular function is unknown. Chitin synthase II is encoded by the CHS2 gene and is essential for growth.

We previously reported three genes, CSD2, CSD3, and CSD4, required for chitin synthesis in vivo. All have been cloned; none are CHS2. Disruption of CSD2 and CSD4 produces strains that are viable despite a 10- to 20-fold reduction in chitin in vivo. To explain the low level of chitin, we measured the chitin synthase II activity. Assay procedures have been described that either include or omit trypsin. We obtained different results in each case. With trypsin, mutant and wild-type membranes gave identical values. When trypsin was omitted, the mutants had no activity relative to a CSD^+ strain. Thus, the CSD genes may be required for post-translational activation of the CHS2 gene product. Alternatively, these genes may define a third chitin synthase. Other phenotypes of the null mutants will be presented.

Sequencing of CSD4 revealed a single open reading frame of 2.1 kb coding for a protein of 77 kd. There is no significant homology to the CHS1 gene product or to any proteins in the NBRF or GENbank databases.

N 203 *RME1*-INSENSITIVE MUTANTS OF *SACCHAROMYCES CEREVISIAE*,
Gautam Kao, Jyoti C. Shah, and Mary J. Clancy, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556. We have screened for mutants which are insensitive to *RME1* repression of sporulation, using the expression of a late gene *'lacZ* fusion as a reporter. Four mutants restore sporulation to *RME1*-blocked cells but do not hypersporulate or deregulate late genes. One such mutation, isolate 125, supports sporulation in *mat*a1/*MAT*α and *MAT*a/*mat*α backgrounds, and allows low expression of late genes in haploids. It is not a cis-acting mutation at *HML*, *HMR* or *MAT*. *RME1* and the gene defined by the 125 mutation act on the same pathway, since double mutants do not show enhanced sporulation in *MAT*-insufficient backgrounds relative to either mutation alone. Because the mutation is dominant, we strongly suspect it to be an allele of *IME1*, a known activator of meiosis and target of *RME1*. If so, the mutant will be informative of the target for mating type vs. nutritional control of this central gene.

N 204 STA1 IS A HAPLOID-SPECIFIC GENE IN S. CEREVISIAE VAR. DIASTATICUS.
A.M.Dranginis, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892.

S. cerevisiae var. diastaticus secretes a glucoamylase which is encoded by the gene STA1. We show that expression of STA1 is regulated by the mating-type locus, MAT. MATa or MATα haploid cells express and secrete high levels of glucoamylase, but MATa/MATα diploid cells produce undetectable levels of the enzyme. Deletion of the MATa1 coding sequences in a diploid cell resulted in high levels of glucoamylase production, whereas deletion of the MATa2 sequences had no effect under any growth conditions. Northern blots of RNA prepared from these cell types show that this regulation occurs at the level of RNA accumulation. Mating-type regulation of STA1 expression is thus effected by the product of MATa1, together with the MATα locus, just as haploid-specific genes (such as HO and RME1) are regulated in S. cerevisiae.

Glucoamylase activity and STA1 RNA levels are also regulated by nutritional conditions. Highest levels of STA1 expression occur in nonfermentable media or in rich media containing starch as the sole carbon source. Addition of glucose to various starch-based media represses glucoamylase synthesis approximately five-fold.

Experiments are currently underway to identify sequences necessary for regulation of STA1 expression by MATa1 and ultimately to identify changes in chromatin structure associated with mating-type regulation of this gene.

N 205 MOLECULAR ANALYSIS OF A MATING TYPE MUTANTS IN NEUROSPORA CRASSA. N.L. Glass and R.L. Metzenberg, Department of Physiological Chemistry, University of Wisconsin, Madison, WI. 53706

Neurospora crassa is a heterothallic filamentous fungus with two mating types, designated as A and a, which occur as alternative, single-copy DNA sequences within a haploid genome. The A and a mating type loci are defined as a 4.4kbp blocks of DNA that have little sequence similarity. Both mating type alleles are necessary for the events associated with mating, but the presence of both in a vegetative heterokaryon produces cell death or inhibited, abnormal growth ("incompatibility"). A series of mating type mutants was identified that are sterile and heterokaryon-compatible (A.J.F. Griffiths, Can. J. Genet. Cytol. 24:167-176, 1982). Sequence analyses of five sterile A mating type alleles revealed frameshift mutations in a 1.0kbp fragment of the 4.4kbp A mating type locus. Sequence and mRNA analyses of the A mating type 1.0kbp fragment indicate that this region would encode a single protein of 313 amino acids; a 59bp intron would be spliced out of the mRNA. Thus, a single product is apparently responsible for both mating type function in the sexual phase and heterokaryon-incompatibility in the vegetative phase. The 1.0kbp A fragment was "RIP"ed (E. Selker, et al. Cell 51:741-752, 1987) and alterations in restriction sites were detected within the 1.0kbp fragment. The phenotype of the "RIP"ed mutants was identical to Giffiths' mutants, i.e., they were sterile and heterokaryon-compatible. The remainder of the A mating type region will be "RIP"ed to define any additional mating or heterokaryon-incompatibility functions associated with the A mating type locus.

N 206 FUNCTION OF YEAST CDC7 PROTEIN IN DNA METABOLISM, **Robert E. Hollingsworth and Robert A. Sclafani, Department of Biochemistry, Biophysics, and Genetics, Univ. of Colorado Health Sciences Center, Denver, Colorado 80220.** The *CDC7* gene of *S. cerevisiae* is indispensible to all aspects of DNA metabolism. Thermosensitive *cdc7* mutants are defective in the initiation of mitotic replication, meiotic recombination, chromosome transmission, and induced mutagenesis (error-prone DNA repair). We have shown that expression of the *CDC7* gene is not regulated in mitosis using measurements of mRNA levels throughout the cell cycle and pedigree segregation lag analysis. In addition, Cdc7 protein production is not altered after UV irradiation or in meiosis, as measured using *E. coli lacZ* as a reporter for *CDC7* promoter function. Thus, activation of Cdc7-catalyzed processes must be at the level of post-translational modification or substrate synthesis. The various mutant alleles affect induced mutagenesis to disparate levels, with some producing hypomutability while others produce hypermutability. Because these mutagenesis phenotypes for the mutant alleles do not correlate with abilities to perform other DNA transactions, the protein may be organized into separate functional domains. Based on sequence similarity to known protein kinases, we have proposed that the Cdc7 protein acts by phosphorylating various target substrates. Kinase assays performed using crude extracts from cells overexpressing the gene sustain this notion, and reveal Cdc7-mediated phosphorylation of endogenous proteins of 48 and 56 kD. The 56 kD substrate may be Cdc7 itself. We have produced Cdc7-specific antibodies, and are using these in our current attempts to purify this protein in order to verify its activity directly. (Supported by NIH grant GM35078).

N 207 YEAST NOVOBIOCIN BINDING PROTEIN, John R. Jenkins, Michael J. Pocklington, and Elisha Orr, Department of Genetics, University of Leicester, Leicester LE1 7RH, England. Novobiocin is a specific inhibitor of bacterial DNA gyrase. The presence of this coumarin results in the inhibition of DNA replication, transcription, transposition and sporulation. Likewise, in eukaryotic cells sensitive to novobiocin, the drug inhibits DNA syntehsis, transcription, differentiation and in addition affects chromatin structure. Though bacterial gyrase and eukaryotic type II topoisomerase exhibit striking structural similarities, the eukaryote enzyme is not the target of novobiocin. Bacterial DNA gyrase can be readily purified by affinity chromatography on novobiocin coupled to a ligand. Yeast novobiocin binding proteins have similarly been analysed. A few proteins bind tightly to the antibiotic, none of which is the nuclear type II topoisomerase. One of these has been identified as a nuclear encoded mitochondrial protein that has been conserved throughout evolution.

N 208 INITIATION OF MEIOSIS IN YEAST REQUIRES THE EXPRESSION OF IME1, A GENE THAT IS NEGATIVELY CONTROLLED BY NUTRIENTS AS WELL AS BY MAT.
Yona Kassir, Milet Treinin, Amir Sherman and Giora Simchen. Department of Genetics The Hebrew University, Jerusalem 91904 Israel.
Initiation of meiosis in the yeast Saccharomyces cerevisiae is determined by two signals: Heterozygosity at the mating type locus, and starvation for nitrogen and glucose. A complex between the MATa1 and MATα2 gene products inhibits the transcription of RME1. The product of RME1 inhibits transcription of IME1 in a medium free of nitrogen and glucose. In a medium without glucose but with nitrogen, RME1 has no effect on the level of IME1 mRNA. In the presence of glucose IME1 transcript is not detected. Using IME1-LacZ fusions we identified seperate sites which are responsible for the above regulation. We shall present data supporting the notion that nutrients regulate IME1 expression via the cAMP dependent protein kinase. Diploids which are homozygous for the $cdc25^{ts}$ mutation (CDC25 is an effector of adenylate cyclase), when shifted to the non-permissive temperature sporulate in rich acetate medium. These diploids also show an immediate increase in the level of IME1 mRNA when shifted to the restrictive temperature. IME1 codes for a protein of 42Kd. It has relatively high levels of Serine and Threonine residues as well as sites which are known to be targets for N- and O- glycosylation.

N 209 MOLECULAR GENETICS OF PHYTOCHELATIN BIOSYNTHESIS IN S. pombe

Lisa Kreppel, Gretchen Scheel, Gary McDonald, Michael Dumars and David W. Ow. Plant Gene Expression Center, USDA/ARS/UC Berkeley, 800 Buchanan St., Albany, CA 94710.

Plants respond to **heavy metal stress** by inducing the synthesis of heavy metal binding peptides known as phytochelatins. Unlike the metallothioneins of animal cells, they are not products of primary translation. They have a unique structure: $(gamma\text{-}glu\text{-}cys)_n\text{-}gly$, with n = 2 to 11. Surprisingly, phytochelatins are also found in the fission yeast, Schizosaccharomyces pombe. Using S. pombe as a model system for studying the synthesis of these peptides that are ubiquitous in the plant kingdom, we have identified mutants with altered production of Cd-phytochelatin complexes. Some of these mutants lack the higher molecular weight form of Cd-phytochelatins that contains sulfide ions. Sulfide containing Cd-phytochelatin complexes are more stable and are believed to confer greater heavy metal tolerance. Another class of mutants is deficient in the synthesis of both high and low molecular weight Cd-peptide complexes. By functional complementation in S. pombe mutants, we have cloned DNA derived from the S. pombe genome that can complement hypersensitive phytochelatin-deficient mutants. Biochemical and genetic characterization of these mutants and their complementating DNA clones will be presented.

N 210 DEVELOPMENT OF A NEW SYSTEM FOR SELECTION OF ASPERGILLUS NIGER TRANSFORMED WITH FOREIGN GENES BY USING THYMIDINE KINASE GENE AS A MARKER AND PRIMARY EXPRESSION OF HBSAG GENE IN ASPERGILLUS NIGER, Hong-Di Liu, Xiao-Jun Wu, Ping-Yan Liang, Institute of Microbiology, Academia Sinica, Beijing, 100080, China, and Xu Tsao, Li Ruan, Institute of Virology, Chinese Academy of Preventive Medicine, 100 Ying Xing Jie, Xuan Wu Qu, Beijing, 100052, People's Republic of China. A new system for selection of transformed Aspergillus niger was reported. In this system, TK^- Aspergillus niger which was formed by homologous recombination of mutated TK gene of vaccinia virus with TK gene of Aspergillus niger can be selected out by adding BUdR in agar plates. More details about screen of TK^+ Aspergillus niger strains, conditions for transformation of Aspergillus niger and selec - tion of transformed TK^- Aspergillus niger have been studied. By using this system, sever- al transformed Aspergillus nigers which contain HBsAg gene drived by a promoter H8 cloned from genomic DNA of Aspergillus niger were isolated. It was demonstrated that HBsAg gene was integrated into the genomic DNA of Aspergillus niger by Southern blot after many pas- sages of spores. ELISA showed that HBsAg was positive in the growth medium(p/n=20). The 22nm particles which were very similar to the HBsAg particles in human serum were found in the growth medium by immunoelectromicroscope. Western blot also gave the specific ba- nds. All these data show that HBsAg gene was expressed in Aspergillus niger and the pro - ducts were secreated into the growth medium. The selection system using TK gene as marker could generally be used to study the expression of foreign gene in Aspergillus niger.

N 211 ATPASE FUNCTION AND GROWTH CONTROL, Norma Neff, Cheng-Kon Shih, and Cynthia Ennulat. Memorial Sloan-Kettering Cancer Center, NY, NY 10021. We have identified three loci which can be altered to give resistance to trifluoperazine (TFP) - resistance in S. cerevisiae. We have cloned and sequenced a gene encoding a dominant mutation to TFP-resistance. The DNA sequence of the TFP1-408 gene predicts a 1031 amino acid protein that has a 70% identity in its first 300 amino acids and in its terminal 400 amino acids with the 67kDa subunit of the Neurospora vacuolar ATPase complex. The entire coding region of the Neurospora vma-1 gene is contained within this yeast gene, however the amino acid identity of these two genes is interrupted in the middle by a 300 amino acid insert in the TFP1 gene. This region of the TFP1 gene has homology with HO, the mating type specific endonuclease (30% identity over 300 amino acids). Mutants at the TFP1 locus show calcium-sensitive growth and have higher rates of calcium uptake. What HO and the TFP1 gene may have in common is a small region of identity that could encode a calcium-binding or responsive domain. Based on its size, amino acid identitities and calcium-sensitive properties of mutant cells, we propose that the TFP1 gene encodes a subunit of a calcium-responsive ATPase. We have identified mutants in yeast which are defective in growth control, protein phosphorylation, and show alterations in the activity of the major plasma membrane ATPase (but do not map to the pma1 locus). These mutants were isolated by their resistance to sodium orthovanadate and have several phenotypes in common with ras mutants, failure to enter stationary phase, sensitivity to heat shock, sporulation on rich media (YPD) and others. Some mutants have 4x higher protein phosphorylation on ser and thr and have altered ATPase activities.

N 212 NSP1 - A GENE ESSENTIAL FOR THE CELL CYCLE IN *SACCHAROMYCES CERREVISIAE,* Ulf Nehrbass, Hildegard Kern, Eduard C. Hurt, EMBL, Meerhofstr.1, 6900 Heidelberg, FRG.

Only a few structural components essential for cell division in the yeast *Saccharomyces cerevisiae* have so far been identified. Using a combined biochemical/genetic approach, a gene coding for a peripheral, nucleo-skeletal-like protein (NSP1) was cloned. Indirect immuno-fluorescence suggests a location of NSP1 at the spindle pole body and the nuclear periphery. The sequence of NSP1 reveals a highly repetitive middle domain not homologous to known proteins and flanking regions which are homologous to cytokeratins and calcium binding proteins. NSP1 is essential for growth and preliminary data suggest that a temperature-sensitve mutant of NSP1 stops growth at the non-permissive temperature showing a cdc-phenotype. Moreover, mutations in the putative, carboxy-terminal calcium binding domain lead to growth arrest. These data support a model in which NSP1 functions as a regulated protein essential for cell division. We are currently trying to substantiate this model by investigation of structure/function relationships and isolation of components interacting with NSP1.

N 213 Casein Kinase II is an Essential Enzyme for Growth in the Yeast, *Saccharomyces cerevisiae.* *Ramesh Padmanabha, Joan L. -P. Chen-Wu and Claiborne V. C Glover. Biochemistry Department, University of Georgia, Athens, GA. 30602.*

Casein kinase II is a multi-potential Ser/Thr kinase that is ubiquitous in all eukaryotes. Although there are a number of known substrates, the exact physiological role of the enzyme is still unknown. The enzyme isolated from most sources consists of a catalytic subunit and a regulatory subunit. The yeast enzyme is unusual in that it has two catalytic subunits. The genes encoding both these subunits have been isolated (*CKA1* and *CKA2* respectively), and the deduced amino acid sequences are about 65% identical to each other and to the catalytic subunit from *Drosophila melanogaster*. Tetrad analysis of null mutations constructed in either the *CKA1* or the *CKA2* gene alone show that the subunits appear to be functionally identical, and the heterozygous haploids do not show any detectable growth defect. Using a homozygous diploid strain disrupted at the *CKA1* locus, a heterozygous strain carrying a disruption at one of the *CKA2* loci was constructed. This diploid (RPG14), which has only a single functional catalytic subunit of casein kinase II is viable and does not appear to posess any detectable growth defect. Tetrad analysis of this strain shows that a *cka1 cka2* haploid is inviable. Microscopic examination of the cells on the plate indicate that they go through 2-3 divisions before arresting. Transformation of RPG14 with a CEN plasmid containing the entire *CKA2* gene, followed by tetrad analysis, shows that the growth defect of *cka1cka2* cells can be rescued with a functional *CKA2* gene. This is the first demonstration in any organism that casein kinase II is an enzyme essential for growth.

N 214 A Type II Ca^{2+}/Calmodulin-Dependent Protein Kinase from Yeast. M.H. Pausch and J. Thorner, University of California, Berkeley, CA 94720. Ca^{2+}/calmodulin (CaM) signal transduction pathways often employ protein kinases as terminal effector elements. To elucidate the function of these enzymes, a Ca^{2+}/CaM-dependent, multifunctional protein kinase (CaM kinase) activity was purified from the yeast *Saccharomyces cerevisiae* by ammonium sulfate precipitation, affinity chromatography on yeast CaM, gel filtration, and Mono Q anion exchange chromatography. The major species present in the most highly purified fractions was a 55 kDa polypeptide. Gel filtration and velocity sedimentation analyses suggest that the 55 kDa species exists in its native state as part of a multisubunit complex. Autophosphorylation of the 55 kDa protein in solution was dependent on both Ca^{2+} and CaM and accompanied by a reduction in its electrophoretic mobility. *In situ* autophosphorylation and ^{125}I-CaM-binding directly demonstrated that the 55 kDa protein possesses both catalytic and CaM-binding sites. The purified enzyme catalyzed the Ca^{2+}/CaM-dependent phosphorylation of a variety of substrates including a peptide analog of a rat brain Type II CaM kinase autophosphorylation site. Thus, this yeast enzyme is a member of the Type II class of Ca^{2+}/CaM-dependent protein kinases and may play an important role in the transduction of Ca^{2+} signals in the yeast cell. Supported by NIH Postdoctoral Traineeship CA09041 (to M.H.P.) and by NIH Research Grant GM21841 (to J.T.)

N 215 A CIRCULAR DNA PLASMID OF SCHIZOPHYLLUM, Carlene A. Raper and J. Stephen Horton, Dept. of Microbiology, University of Vermont, Burlington, Vt. 05405. We have discovered a circular DNA plasmid of 5 kb in strains differentiating the specialized hook-cells of the so-called A developmental sequence controlled by the A mating-type genes in Schizophyllum. The plasmid has not been found in coisogenic strains not differentiating hook-cells. The cloned plasmid is being tested for biological function in transformation experiments. We also have isolated clones of Schizophyllum DNA homologous to the yeast 2 micron circle. Whether or not these relate to the Schizophyllum plasmid DNA is not yet known.

N 216 DELETION ANALYSIS OF A PROTEIN KINASE REQUIRED FOR MATING IN YEAST, N. Rhodes, B. Errede, University of North Carolina, Chapel Hill, NC 27599-3290.

The yeast Saccharomyces cerevisiae can exist as three cell types: a, α, or a/α. The ability of a and α cells to mate with each other to form an a/α diploid cell requires cell-type recognition and intracellular signaling. The STE11 gene product is one component of the signaling pathway. In the absence of a functional STE11 gene product haploid cells of either cell-type are unable to mate, unable to respond to the signal initiated by binding of mating pheromone to cell surface receptor, and produce reduced amounts of mRNA for cell-type-specific genes. The STE11 gene product is a protein kinase. We have applied directed deletion analysis to define domains of the STE11 protein that are required for possible regulation and catalytic activity. The carboxyl terminus of STE11 contains the catalytic domain and has been shown to be essential for mating and transcriptional activation of cell-type-specific reporter genes. We are currently investigating the requirement of the amino terminus of the STE11 kinase for mating, response to pheromone, and transcription activation.

N 217 MSI1: A NOVEL NEGATIVE REGULATOR OF THE RAS/cAMP PATHWAY IN SACCHAROMYCES CEREVISIAE, Rosamaria Ruggieri, Kazuma Tanaka[1], Masato Nakafuku[2], Yoshito Kaziro[2], Akio Toh-e[1], and Kunihiro Matsumoto, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304; [1]Department of Fermentation Technology, Hiroshima University; and [2]Institute of Medical Science, University of Tokyo. Recently we identified a gene, IRA1, whose product is an inhibitor of the RAS functions in Saccharomyces cerevisiae (1). Disruption of IRA1 causes sensitivity to nitrogen starvation and to heat shock, and deficiency in sporulation in homozygous diploids. Making use of the heat shock sensitivity of the ira1 mutation, we have isolated a gene, MSI1, that suppresses this phenotype when expressed in cells on a high copy number plasmid. Besides the heat shock sensitivity, the MSI1 gene suppressed also the sporulation defect of ira1 and RAS2^{val19} cells. MSI1 was not able to suppress similar phenotypes of bcy1 cells. Moreover, the high cAMP levels observed in RAS2^{val19} were reduced by overexpression of the MSI1 gene. THe MSI1 nucleotide sequence revealed an open reading frame encoding a putative protein of 422 amino acids, that contains some homology to mammalian transducin β. Our data suggest that the MSI1 protein is a negative regulator of the RAS2 mediated induction of cAMP in yeast.

(1) Tanaka, K., Matsumoto, K., and Toh-e, A. IRA1: an inhibitory regulator of the RAS/cAMP pathway in Saccharomyces cerevisiae. M.C.B. In press.

N 218 Functional Similarity of Mammalian Growth-Associated H1 Kinase and the *CDC28* protein kinase of *Saccharomyces cerevisiae*. **Robert A. Sclafani[1], Thomas A. Langan[2], Robert E. Hollingsworth[1], Jean Gautier[2], James L. Maller[2], Paul Nurse[3] and Michael Klein[1]. Departments of Biochemistry, Biophysics and Genetics[1] and of Pharmacology[2], University of Colorado Health Sciences Center, Denver, CO 80262; University of Oxford[3], United Kingdom.** Mammalian growth-associated growth H1 kinase, an enzyme whose activity is sharply elevated as cells enter mitosis, phosphorylates specific sites in H1 histone. We show that the *CDC28* protein kinase of *Saccharomyces cerevisiae* catalyzes the phosphorylation of these same sites, as indicated by the patterns of chymotryptic and tryptic phosphopeptides derived from phosphorylated mammalian H1 histone using both wild-type and *cdc28* mutant extracts. Regulation of the yeast enzyme is also similar because a three-fold increase in enzyme activity occurs in cells synchronized at mitosis using nocadazole or a *cdc16* mutant. Antibodies specific for a peptide sequence conserved in the *CDC28* protein, its homolog in *Schizosaccharomyces pombe* and in human cells recognize the 34Kd products of these homologous genes (Lee and Nurse-1987, Nature 327, 31). The antibodies specifically immunoprecipitate rat growth-associated H1 histone kinase activity and are immunoreactive with a 32-34Kd protein present in partially purified preparations of this enzyme. Because the yeast enzyme is required for entry into mitosis and is similar in structure, activity and regulation to the mammalian enzyme, this type of protein kinase has an essential function in the entry of cells into mitosis by a mechanism that is conserved in all eukaryotes. These findings suggest that yeast cells may have an H1-like protein which we are currently investigating and hope to present supporting evidence. (Supported by ACS Inst. grant)

N 219 STEROID-HORMONE REGULATION OF THE ACHLYA 85kD HEAT-SHOCK PROTEIN, WHICH IS A COMPONENT OF THE ACHLYA STEROID-RECEPTOR. Julie C. Silver[1], Robert Riehl[3], David O. Toft[2], and Shelley A. Brunt[1]. [1]Department of Microbiology and Division of Life Sciences, University of Toronto, Scarborough, Ontario, Canada, M1C 1A4; [2]Department of Cell Biology, Mayo Clinic and Foundation, Rochester MN, 55905; [3]Department of Ob-Gyn, University of Texas Health Science Center, San Antonio, TX 78284. The steroid hormone antheridiol, regulates sexual development in the fungus Achlya. Analysis of in vitro translation products of RNA isolated from control, heat-shocked or hormone-treated cells demonstrated an increased accumulation of mRNA encoding a similar 85 kDa protein in both the heat-shocked and hormone-treated cells. A monoclonal antibody, AC88, which recognizes the non-hormone binding component of the Achlya steroid receptor, recognized both the hormone-induced and heat-shock-induced 85 kDa proteins in the in vitro translation products. These results suggest that an identical 85 kDa protein is independently regulated by the steroid hormone antheridiol and by heat shock and that this protein is part of the Achlya steroid-hormone-receptor complex. Southern hybridization analysis, using a Drosophila hsp83 clone, suggests that there are at least two putative hsp85 genes in the Achlya genome. Northern blot analyses using the Drosophila probe, indicate that a mRNA species of similar size is substantially enriched in both heat-shocked and hormone-treated cells. Whether these putative Achlya hsp85 genes are each regulated by both stress and steroid hormone remains to be determined.
(Supported by NSERC Canada)

N 220 *ASPERGILLUS NIDULANS* CONTAINS GENES HIGHLY HOMOLOGOUS TO THE MAMMALIAN *RAS* PROTOONCOGENE FAMILY, Tapan Som and James R. Broach, Department of Biology, Princeton University, Princeton, NJ 08544. A wide variety of eukaryotic organisms, including yeasts, fruit flies, slime molds, sea snails, and mammals, contain a highly conserved family of *ras* genes, which encode small monomeric proteins that bind and hydrolyze GTP. In mammals, the products of these genes are apparently involved in signal transduction, coupling mitotic growth to the presence of various serum growth factors. In *Saccharomyces cerevisiae*, *RAS* genes modulate adenylate cyclase activity in response to carbon source and, possibly, other nutrients. In *Schizzosaccharomyces pombe*, mutations of the single *RAS* gene alters the cell's ability to access the conjugation/meiotic pathway in response to starvation. Given the diverse roles of the *ras* genes in different organisms, we felt that examination of the function served by *ras* homologues in another experimentally tractable system could prove informative in an attempt to identify unifying principles of *ras* activity. Accordingly, we have initiated a study of *ras* gene homologues in *Aspergillus nidulans*. Using low stringency hybridization, we have shown that *A. nidulans* contains two genes closely related to the mammalian H-*ras*. We have cloned and sequenced one of these genes, which we have designated *A-ras1*. The DNA sequence of the gene identifies an uninterrupted open reading frame that would yield a protein of 23 Kd. The protein sequence exhibits 70% identity with human cellular H-*ras*, conserving all of the domains shown to be essential for function in the mammalian protein. Experiments are in progress to replace the chromosomal *A-ras1* gene with a disrupted allele and to introduce a copy of the gene bearing an activating mutation We are also in the process of isolating and characterizing the second *ras* gene.

N 221 **CELL CYCLE PROGRESSION AND ASYMMETRIC CELL DIVISION IN INDIVIDUAL CELLS OF BUDDING YEAST**, Friedrich Srienc, David E. Block, Phillip D. Eitzman and Jeffrey D. Wangensteen, Institute for Advanced Studies in Biological Process Technology, University of Minnesota, St. Paul, MN 55108.
To study the distribution of cell propagation through the cell cycle and to investigate the distribution of cell partitioning at cell division we have interfaced a flow cytometer with a dual trace digitizing oscilloscope and a microcomputer permitting automatic acquisition of pairs of waveforms generated by slit scanning of individual yeast cells. This experimental approach yields in addition to cellular composition also information on low resolution spatial distribution of specific properties within individual cells. Because the measurement takes less than a second large numbers of cells can be processed and the distributions of the properties can be established. We have used light scattering measurements to determine cell size, fluorescence measurements to determine DNA content, and immunofluorescence to quantify transient expression of specific proteins in individual cells of growing *Saccharomyces cerevisiae* cell populations. Quantitative parameters that are obtained at the individual cell level include mother cell size, the size of the growing cell bud, the position of the nucleus within a cell, the DNA content within the nucleus, individual stages of nuclear division, and partitioning of cellular components at cell division. Preliminary data obtained with exponentially growing cell populations have shown that at a given DNA content mother cell portions of budding cells show a significant size distribution. While the average size of the mother cell portions remains constant throughout the cell cycle, the cell bud increases in size as function of cell cycle position.

N 222 MOLECULAR GENETICS OF PHYTOPHTHORA MEGASPERMA f.sp. GLYCINEA
Wendy Thompson, Yuxin Mao, Areelak Kashemsanta and Brett M. Tyler.
Department of Plant Pathology, University of California at Davis, Davis, CA 95616.

The oomycetes, including Phytophthora, are a major group of fungal plant pathogens infecting a very wide range of hosts. Phytophthora megasperma f.sp. glycinea (Pmg) is a major pathogen of soybeans causing damping-off of seedlings and a root and stem rot of mature plants. There are at least 10 different soybean major resistance genes against Pmg and these are dominant. In the fungus which is diploid there is some genetic and biochemical evidence to indicate the presence of avirulence genes which correspond gene-for-gene with the host major resistance genes.

In preparation for trying to clone these presumptive avirulence genes we have characterised the genome of Pmg by Tm and Cot analysis. Our present evidence indicates that the Pmg genome has a complexity of 100Mb and contains 70% moderately repeated sequences. The GC content of the genome is 52%.

We are also developing a transformation system for introducing cloned DNA into Pmg. We have constructed bleomycin, chloramphenicol and hygromycin resistance genes for Pmg using the respective E.coli resistance genes in combination with transcriptional sequences from the hsp70 heat shock gene of Bremia lactucae. We are attempting to introduce these genes into Pmg protoplasts by treatment with PEG and $CaCl_2$ and electroporation.

N 223 SUPPRESSION OF THE MATING RESPONSE IN YEAST BY SCG1-Gα HYBRIDS, Donald J. Tipper, Yoon-Se Kang, J. Kurjan*, and J.F. Stadel**, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655, *Department of Biological Sciences, Columbia University, NY, NY 10027, **Molecular Pharmacology, Smith Kline and French Laboratories, King of Prussia, PA 19406. The S. cerevisiae SCG1/GPA1 gene product, Gαγ, resembles mammalian Gα proteins and negatively regulates the G1 arrest response to mating pheromones. On the basis of genetic analysis and by analogy with mammalian systems, it is hypothesized that Gαγ-GDP binds to the yeast Gβγ components, STE4 and STE18, which are the proximal positive effectors of G1 arrest. Conversion to Gαγ-GTP by interaction with occupied receptor causes release of Gβγ and arrest ensues. It was previously shown that Gαs, expressed on CUP1, would weakly suppress the growth arrest phenotype of an SCG1-null mutation. We have extended these analyses using promoters of intermediate (GAL-1) and high (PGK) efficiency and a series of mammalian-yeast hybrid Gα proteins in order to assess the role of different Gα domains in binding to effector (Gβα) and receptor. Only the native Gαγ interacted functionally with receptor. Overexpression resulted in a sterile phenotype, presumably by overwhelming the capacity for receptor-mediated GDP-GTP exchange. The ability of other constructs to suppress growth arrest in an SCG1-null mutant correlated with suppression of mating response in an Scg1$^+$ host. These phenotypes, presumably reflecting ability to titrate yeast Gβγ, were efficiently expressed by hybrids comprising SCG1 residues 1-330 and C-termini from Gαs, Gαi or Gαo. Hybrids with SCG1 C-termini were very inefficient.

N 224 GENETIC CONTROL OF NUCLEAR DIVISION IN YEAST IN RESPONSE TO DNA DAMAGE, Ted A. Weinert and Leland. H. Hartwell, Department of Genetics, SK50,University of Washington, Seattle, WA. 98195.

In studies of mitotic cell division in S. cerevisiae we reported that DNA damage induces RAD9-dependent G2 delay (Science 241,317). The arrest of temperature sensitive cell division cycle mutations (cdc) was investigated; arrest of 3 cdc mutants that arrest in G2 is RAD9-dependent (cdc17, 9, and 13 encoding DNA polymerase I, DNA ligase, and function unknown). The viability at the restrictive temperature of these cdcxrad9 double mutants is dramatically reduced, as expected if cell division proceeds with damaged chromosomes. Arrest of other cdc mutants is unaffected, including other mutations that lead to G2 arrest and arrest in S phase. Our analysis suggests RAD9-dependent control is DNA damage and G2 phase specific.

The RAD9 gene contains an open reading frame encoding a protein of 149 kD bearing no similarities to other known proteins. Cells induced to G2 delay due by DNA damage show an alteration of RAD9 expression at transcriptional or translational levels. Strains bearing complete deletions are viable but lose chromomsomes spontaneously at 5-10 fold higher rate demonstrating a role for G2 delay in fidelity of chromosome transmission.

We have now isolated mutations in 2 additional complementation groups whose phenotypes are similar to rad9. Study of these additional mutations as well as attempts to identify homologes to RAD9 in mammalian cells are underway.

N 225 POLYPEPTIDES THAT INTERACT WITH THE CDC28 PROTEIN KINASE OF *SACCHAROMYCES CEREVISIAE*, Curt Wittenberg, Jeffrey A. Hadwiger, Helena E. Richardson, Miguel de Barros Lopes and Steven I. Reed, Department of Molecular Biology, MB-7, Research Institute of Scripps Clinic, 10666 No. Torrey Pines Rd., La Jolla, CA 92037

The Cdc28 protein kinase is required for initiation of new cycles of cell division in the budding yeast, *S. cerevisiae*. In an attempt to identify genes encoding polypeptides that interact with the Cdc28 protein kinase, dosage suppressors of temperature-sensitive mutations in the *CDC28* gene were isolated. This approach resulted in the isolation of three previously unidentified genes.

One of these genes, *CKS1*, encodes an 18 kd polypeptide having 67% amino acid homology to the product of the *suc1*$^+$ gene of the fission yeast, *Schizosacharomyces pombe*. Cells carrying a disruption of this gene are inviable and, like *cdc28* mutants, arrest during the G1 interval of the cell cycle. The 18 kd *CKS1* gene product has been identified in yeast extracts using anti-*CKS1* serum and has been shown to be associated with active forms of the Cdc28 protein kinase complex.

CLN1 and *CLN2*, isolated using the same approach, encode homologs of cyclins, a family of proteins recently shown to be required for cell division in eukaryotes. The predicted products of the *CLN1* and *CLN2* genes are 57% homologous to each other. Although cells carrying a single disruption of either of these genes are unaffected, disruption of both genes results in cells that, while viable, are temperature-sensitive for proliferation. The lack of lethality may be explained by the existence of additional family members. A dominant allele produced by truncation of 30% of the *CLN2* ORF exhibits reduced cell size, partial mating pheromone resistance and the inability to respond appropriately to nitrogen limitation. The duration of the G1 interval in these cells is markedly diminished. Further characterization of this allele and the nature of the interaction between the Cdc28 protein kinase and the *CLN* gene products is in progress.

N 226 DNA TOPOISOMERASE I MUTANTS IN YEAST, Ling-Chuan C. Wu[*+] and James C. Wang[*], [*]Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138 and [+]Division of Public Health and Environmental Laboratories, New Jersey Department of Health, Trenton, NJ 08625

Type I topoisomerases transiently cut and reseal one DNA strand. We have designed a strategy to isolate DNA topoisomerase I mutants in Saccharomyces cerevisiae that cleave DNA but are unable to reseal the breaks. This mode of action will effectively mimic the action of the antitumor drug camptothecin, which inhibits DNA topoisomerase I in vitro by stabilizing a protein-DNA adduct with a covalent linkage of the enzyme to the 3'-phosphate of DNA. Presumably, this mode of action is responsible for the drug's cytotoxicity and inhibition of nucleic acid metabolism in vivo. We assume that when the gene is expressed, this class of mutant would be dominant lethal, and thus convert the normally dispensable topoisomerase I gene into a dominant lethal allele.
To screen for such mutants, we mutagenized the topoisomerase I structural gene randomly, and then expressed it from a very weak, yet inducible, mutant GAL1 promoter. This pool of mutagenized DNA was transformed into either a repair-deficient rad52 haploid host or a wild-type diploid strain. In both screens 2 out of a total of 10^4 cells behaved as apparent galactose-inducible, dominant-lethal mutants. We are in the process of characterizing these possible mutants.

N 227 Schizosaccharomyces pombe GENE FOR ADENYLATE CYCLASE AND THREE OTHER cAMP-RELATED GENES INVOLVED IN COMMITMENT TO MEIOSIS, Masayuki Yamamoto, Tatsuya Maeda, Asako Sugimoto, Nobuyoshi Mochizuki, Keiko Hirabayashi, Yoshinori Watanabe and Yuichi Iino, Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, PO Takanawa, Tokyo 108, Japan
Cyclic AMP added exogenously inhibits meiosis in S. pombe. A critical target of this inhibition is expression of the mei2 gene, which is essential for initiation of meiosis. This gene is normally induced by nitrogen starvation and quantitative measurements showed that the amount of intracellular cAMP is reduced under such conditions. Thus, it is possible that cAMP mediates a nutritional signal in meiotic control in S. pombe. To clarify this signal transduction pathway, we cloned the gene for adenylate cyclase (acy1) from the fission yeast. The deduced acy1 gene product has 60% amino acid identity with that of S. cerevisiae in the C-terminal catalytic domain, while they are less homologous in the other regions. We cloned three more S. pombe genes which are related to the pathway. Two of them, named pac1 and pac2, each inhibit expression of mei2 and hence meiosis if introduced into S. pombe at a high copy number. The third gene, steX, is essential for induction of mei2 transcription under nitrogen starvation. Thus, cells defective in steX or cells overexpressing either pac1 or pac2 mimic the physiological effects of a high concentration of cAMP. The deduced products of pac1, pac2 and steX, however, showed no significant homology with obvious candidates such as cAMP-dependent protein kinase or phosphodiesterase. Analysis of epistasis among these genes and characterization of their gene products are in progress.

Transcriptional Control Mechanisms

N 300 PHOTOFOOTPRINTING IN VIVO REVEALS NOVEL FEATURES OF GAL1 TRANSCRIPTION ACTIVATION, Axelrod, J.D. and Majors, J., Dept. of Biochemistry, Washington U. Sch. of Med., St. Louis, MO 63110. A new method for photofootprinting in vivo has been used to examine the mechanism by which the UAS transmits its signal from a distance during transcription activation of the GAL1 gene of S. cerevisiae. Photoproducts in the DNA of UV-irradiated cells are detected by multiple rounds of annealing and extension using Taq polymerase. Changes in photoproduct patterns generated under different regulatory conditions reflect changes in DNA conformation caused by proteins interacting with the DNA either locally or at a distance. To address the question of how a signal is propagated from the UAS to promoter sequences downstream, we have examined a photofootprint between the UAS and the TATA box of GAL1, which is in a sequence thought not to be required for transcription activation. The footprint appears strictly as a function of activation of the UAS by growth on appropriate carbon sources or by trans-acting regulatory mutations. The footprint is seen upon activation even when the TATA box is mutated and transcription is nearly abolished. Furthermore, upon substitution of truncated GAL4 proteins (the trans-activator) retaining either wild type or synthetic activation domains, the footprint is seen only if the substituted protein is competent to activate transcription. We are currently testing the effects of substituting heterologous UASs to ask if the footprint depends on the specific UAS or is a general feature of activation. In addition, we have altered the sequences where the footprint is observed and are asking if the footprint results from a sequence specific contact or results indirectly from a long range conformational effect, perhaps due to looping.

N 301 CDP1, A ZINC-FINGER CONTAINING GENE INVOLVED IN THE EXPRESSION OF THE YEAST COPPER METALLOTHIONEIN GENE. Lawrence W. Bergman and Barbara K. Timblin, Department of Chemistry, Clippinger Labs, Ohio University, Athens, OH 45701
We have isolated a single copy gene, CDP1 (CUP 1 DNA binding Protein) by screening a yeast-λgt11 Library with a labeled DNA fragment containing the upstream activator sequences of the yeast copper metallothionein gene. Southern analysis of a chromosome blot indicates that this gene maps to chromosome XII. Preliminary DNA sequence analysis reveals that CDP1 contains a Zn-finger motif homologous to the consensus Zn finger region of the Xenopus TFIII A protein. Studies concerning the expression and function of CDP1 gene will be discussed.

N 302 PHO2 AND GCN4 PROTEINS BIND TO THE YEAST TRP4 PROMOTER IN A MUTUALLY EXCLUSIVE MANNER, Gerhard H. Braus and Hans-Ulrich Mösch, Microbiological Institute, Swiss Federal Institute of Technology (E T H), CH-8092 Zürich, Switzerland
Two regulatory proteins, PHO2 and the general control regulator GCN4, bind in vitro to the promoter of the tryptophan biosynthetic TRP4 gene; the TRP4 gene product catalyses the phosphoribosylation of anthranilate. PHO2 binds specifically to the TRP4 promoter, but does not bind to any other TRP promoter. PHO2 and GCN4 proteins bind in a mutually exclusive manner to the same sequence, UAS_1, one of two GCN4 binding sites in the TRP4 promoter. UAS_1 is the major site for GCN4-dependent TRP4 activation. The second GCN4 binding site, UAS_2, interacts with GCN4 alone. PHO2 binding interferes with the general control response of TRP4 under low phosphate conditions and simultaneous amino acid starvation and thus the PHO2 regulatory protein connects phosphate metabolism and amino acid biosynthesis in yeast. The GCN4 protein mediates the response of the transcriptional apparatus to the environmental signal 'amino acid limitation', while PHO2 seems to be the phosphate sensor that adjusts the response to the availability of phosphate precursors.

N 303 TRANSCRIPT ANALYSIS OF THE ASPERGILLUS NIDULANS REGULATORY GENE areA
Mark X. Caddick*, P. Hooley# and H.N. Arst Jr+. *Department of Genetics and Microbiology, University of Liverpool, P.O. Box 147, Liverpool, U.K. and +R.P.M.S., Hammersmith Hospital, DuCane Road, London, W12 ONN, U.K.
areA encodes a positive acting wide domain regulatory gene which mediates nitrogen metabolite repression in A. nidulans, and has also been implicated in oxygen repression of the pyrimidine salvage pathway. areA has been extensively characterised using classical genetics (Wiame, J.-M., Grenson, M. and Arst, H.N. (1985) Adv. in Microb. Physiol. 26; 1-88). More recently areA has been cloned (Caddick et al. (1986) EMBO J. 5; 1087-1090) and sequenced by us (unpublished). We have employed a variety of areA alleles including chain termination and other null mutants, an inversion, and translocations, to characterise the regulation of the areA transcript. To do this we have utilized Northern and dot blots, nuclease mapping, primer extension and cDNA clones. We conclude that:

i) The level of the areA transcript is highest under conditions of ammonium derepression.
ii) Functional areA product is not fully required for this regulation.
iii) The regulation of the areA transcript is dependant on sequences at both the 5' and 3' end of the gene.
iv) A number of distinct transcripts are produced; their relative proportions vary with the growth conditions.
v) The areA product is involved in regulating the variation in transcripts.

N 304 AN UPSTREAM REPRESSION SITE OF THE YEAST ENOLASE GENE *ENO1*,

Andrew A. Carmen, Alan E. Pepper and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California; Davis, CA 95616

There are two enolase genes (*ENO1* and *ENO2*) per haploid genome of the yeast *Saccharomyces cerevisiae*. *ENO2* expression is induced 20-fold in cells grown in the presence of glucose versus gluconeogenic carbon sources while *ENO1* is constitutively expressed in these carbon sources. Both genes contain a complex enhancer-like region (upstream activation sites, UAS). These UAS regions are required for expression of each gene and contain sequences which mediate glucose dependent induction. The *ENO1* gene also contains an upstream repression site (URS) located approximately 210bp upstream from the initiation codon. This URS region prevents glucose dependent induction of *ENO1* and is essential for the constitutive expression of the *ENO1* gene. Sequences required for URS function have been localized by deletion mapping analysis. Gel retention and footprinting analysis indicate that proteins bind specifically to this region. At least two distinct complexes are observed when a URS containing DNA fragment is incubated with yeast S100 extracts. Both phosphocellulose and MonoS (FPLC) chromatography have been used to separate binding activities. MonoS chromatography has resolved three distinct URS binding activities. These observations suggest that there are specific *trans*-acting regulatory elements which interact with URS.

N 305 RAP1 IS A COMPONENT OF A COMMON TRANSCRIPTIONAL CONTROL CIRCUIT OF YEAST GLYCOLYTIC GENES, Alistair Chambers, Clive Stanway[1], Jimmy Tsang[1], Hiroshi Uemura[2], Alan Kingsman[1] and Susan Kingsman[1], [1]Dept. of Biochemistry, Oxford University, Oxford OX1 3QU, [2]National Chemistry Laboratory for Industry, Kaguku Gijutsu, Kenkyusho, Tsukuba Research Center, Yatabe, Ibaraki 305, Japan

The UAS of the phosphoglycerate kinase gene (PGK) is composed of three distinct functional elements which have been defined by deletion analysis and the construction of a minimal PGK promoter. One of these elements, the activator core (AC) sequence is homologous to the consensus RAP1/GRF1/TUF factor binding site; which we have confirmed by methylation interference studies. We have shown that the protein which binds to the AC sequence is RAP1 by using an antibody raised against purified RAP1. Furthermore we have observed that the UASs of other yeast glycolytic genes have features in common with the PGK UAS, including RAP1 consensus binding sites. As a result we have demonstrated that RAP1 also binds to the PYK and ENO1 UASs. These data indicate that RAP1 is a component in the control of glycolytic gene transcription.

N 306 ALTERING THE DNA BINDING SPECIFICITY OF GAL4 REQUIRES SEQUENCES ADJACENT TO THE ZINC FINGER, J. Chris Corton and Stephen Johnston, Dept. of Botany, Duke Univ., Durham, N.C. 27706

The transcriptional activator GAL4 from Saccharomyces cerevisiae is in a class of regulatory proteins which have one zinc finger within their DNA binding domains. It has been proposed that the DNA binding region in this class of proteins has two functional domains: a cysteine-rich region containing the zinc finger which interacts nonspecifically with the DNA and a region immediately adjacent to the finger necessary for specific recognition of the upstream activating sequence (UAS) (Salmeron and Johnston, 1986). To test this hypothesis, we have replaced increasing portions of the zinc finger and the predicted specificity region of GAL4 with the analogous sequences of another yeast activator protein, PPR1. These data demonstrate that 1) the finger region of GAL4 can be replaced with the PPR1 finger region without changing the DNA binding specificity, 2) high Gal^+ activity of the GAL4 hybrid containing the PPR1 zinc finger region requires one GAL4 amino acid (Lys23) within the PPR1 zinc finger, and 3) sequences immediately flanking the finger region are required for specific recognition of the UAS.

N 307 THE SILENCER ELEMENT FROM HMR IS FUNCTIONAL OUTSIDE THE SILENT MATING-TYPE LOCUS, C. Davies*, M. Hoekstra†,F. Heffron†, and B. Errede*, *University of North Carolina, Chapel Hill, NC 27599-3290, †Research Institute of Scripps Clinic, La Jolla, CA 92037.
Transcription from the silent mating-type loci, HML and HMR, in S. cerevisiae is repressed by factors acting through the adjacent E site, or "silencer" element. Previous studies have shown that transcription from heterologous promoters and genes placed at HML and HMR is repressed, and that this repression can be controlled by the trans-acting products of the SIR genes.

Here we demonstrate that a 500-bp fragment containing the E site can also function to repress transcription when situated away from the silent mating-type loci. Through a Tn3-based transposon mutagenesis approach, we have placed transcription of ADE1 on chromosome I under the control of a transposon-borne E site. A conditional ADE1 phenotype is observed when the E site is brought under the control of ste9, a temperature sensitive allele of SIR4.

N 308 ENHANCEMENT OF ASPERGILLUS NIDULANS TRANSFORMATION BY THE ans1SEQUENCE IN CIS AND TRANS,Agnieszka Dzikowska,Piotr Weglenski,Department of Genetics,Warsaw University Al.Ujazdowskie 4,00-478 Warsaw,Poland.The A.nidulans ans1 sequenceisolated by Ballance and Turner (1985) was shown to enhance the efficiency of transformation when introduced into vectors containing argB or trpC genes.Increased efficiency of transformation is also observed when ans1 is present on a second cotransforming plasmid without a selectable marker. In an attempt toexplain the ans1 trans activity we have performed hybridization analysis of some cotransformants which arose after transformation with two different plasmids based on argB or trpC selectable markers with or without ans1.In two of three cotransformants obtained after cotransformation with one plasmid containing ans1 there was probably a linked integration of both plasmids.We obtained bands which hybridize simultaneously with argB and trpC probes.This could result from a recombination event before integration.
Refrences:BallanceDJ,Turner G (1985)Gene 36:321
Cullen D et al.(1987)Nucleic Acids Res 15:9136

N 309 HERPES VP16 EFFECTS ON YEAST POLYMERASE II TRANSCRIPTION IN VITRO, Aled M. Edwards, Raymond Kelleher, Daniel I. Chasman, Peter Flanagan, Neal F. Lue, and Roger D. Kornberg, Department of Cell Biology, Stanford University, School of Medicine, Stanford, California 94305.

The rate of transcription initiation can be increased by "activator" proteins that bind to DNA elements (termed upstream activating sequences, or UASs, in yeast) near a promoter. Recently, a potent activator has been described in which the DNA binding region of GAL4, a UAS_{GAL}-binding protein, is fused to an acidic region of VP16, a herpes simplex virus-encoded transcriptional activator. This GAL4-VP16 fusion protein was shown to stimulate transcription from promoters with GAL4-binding sites in mammalian cells. It was further shown that GAL4-VP16 can markedly increase the amount of UAS_{GAL}-dependent transcription in a yeast nuclear extract. We have now characterized effects of GAL4-VP16 on transcription in vitro with respect to the following: 1. Dependence of activation on distance between UAS_{GAL} and the promoter; 2. Effects of GAL4-VP16 on transcription from a promoter with a different UAS, and upon basal level (UAS-independent) transcription; and 3. Stage in the reaction when GAL4-VP16 exerts its effects. Our findings bear on the question of whether GAL4-VP16 interacts with a component of the transcription apparatus, and whether GAL4-VP16 can sequester this component, making it unavailable for the transcription of genes without GAL4-binding sites.

N 310 REGULATORY MUTANTS THAT ALLOW UNSCHEDULED EXPRESSION OF GENES IN TWO SEPARABLE PATHWAYS IN MEIOTIC DEVELOPMENT, Rochelle Easton Esposito, Randy Strich, and Michael R. Slater, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

Regulatory mutants have been isolated that derepress genes that function in two separable pathways required for normal meiotic development in *Saccharomyces cerevisiae*. These mutants allow the unscheduled expression of two meiosis-specific genes during vegetative growth in haploid cells. One of these genes, *SPO13*, is necessary for proper chromosome segregation during Meiosis I, while the other, *SPO11*, is involved in meiotic recombination. The mutations are recessive and fall into five complementation groups designated *ume1*, *ume2*, *ume3*, *ume4*, *ume5* (for *U*nscheduled *M*eiotic Gene *E*xpression). Not only do the *ume* mutants derepress the transcription of the two meiosis-specific genes described, they also interfere with meiosis and spore formation itself (ascus production is inhibited 100-fold in either *ume4* or *ume5*), suggesting that the wild-type alleles of some *UME* genes are essential for normal meiosis. The mutant *ume* phenotypes indicate that *UME* genes are part of a regulatory cascade acting downstream of both the cell type and nutritional controls of meiosis.

N 311 TRANSCRIPTIONALLY ACTIVE YEAST 5S CHROMATIN: IN VIVO AND IN VITRO. Sara J. Felts, P. Anthony Weil and Roger Chalkley, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232. We have developed the yeast 5S rRNA gene into a model system for the assembly of transcriptionally active chromatin. In vivo studies reveal that the endogenous 5S genes, as well as centromeric 5S maxigenes, are associated with an array of phased nucleosomes. Indirect end-labeling experiments to map protein-DNA interactions are consistent with the conclusion that a transcription complex is associated with the internal control region of the gene while nucleosomes are phased 5′ and 3′. In vitro reconstitution studies show that TFIIIA is not sufficient for the formation of active 5S chromatin. Instead, a stable transcription factor complex, consisting of TFIIIA, TFIIIB, and TFIIIC must be formed prior to the addition of histones in order for the fully-assembled template to be transcribed by RNA polymerase III. In addition, chromatin transcription studies with 5S maxigenes demonstrate that RNA polymerase III is able to transcribe through two or three nucleosomes but is unable to transcribe through a longer array of nucleosomes. This reflects its behavior on the same 5S maxigenes in vivo.

N 312 MOLECULAR ANALYSIS OF GLUCOSE REPRESSION OF THE GAL1 PROMOTOR. Jeff Flick and Mark Johnston, Dept. of Genetics, Washington University, St. Louis, MO 63110.

Expression of the GAL1 gene in S. cerevisiae is strongly repressed by growth on glucose. We have observed two sites within the GAL1 promotor that are subject to glucose repression. First, glucose inhibits the ability of GAL4 protein to bind to the UASgal. Second, a promotor element downstream of the UASgal confers glucose repression independently of GAL4. We designate this downstream element URSgal and have attempted to delimit the DNA sequences involved. A 97bp fragment containing the GAL1 sequences between UASgal and the TATA box is sufficient to confer repression. However, mutant promotors deleted for small (20bp) segments that span this region are still subject to glucose repression. This suggests that there are multiple sequences within this region that confer repression and that no single sequence is essential.

We are also attempting to identify the cellular factors which act upon the URSgal. We have identified two genes, GRR1 and REG1, which are required for repression. These genes may be involved in an early step of glucose repression because they are also required for the repression mechanism that inhibits GAL4 DNA binding. Currently, we are screening for mutations which specifically relieve repression of the URSgal.

N 313 **CHARACTERIZATION OF THE *ASPERGILLUS NIGER* GLUCOAMYLASE PROMOTER.** Tim Fowler and Mick Ward. Genencor Inc., South San Francisco, CA, 94080

Glucoamylase (E.C. 3.2.1.3.) catalizes the reaction of starch and other malto-oligosaccharides to glucose. It has been noted that glucoamylase expression proceeds at high levels in the presence of soluble starch, however expression is reduced in the presence of glycerol or xylose.

We have investigated the functional organization of the promoter region for the glucoamylase gene of *A. niger* by sequential deletion of DNA sequences 5' to the mRNA start site.

The effects of the deletions on regulation and expression of glucoamylase, when reintroduced into a strain deleted for the host glucoamylase, will be presented.

N 314 THE CYS-3 MAJOR SULFUR REGULATORY GENE OF NEUROSPORA CRASSA ENCODES A PROTEIN WITH A PUTATIVE LEUCINE ZIPPER STRUCTURE, Ying-Hui Fu and George A. Marzluf, Department of Biochemistry, Ohio State University, Columbus, OH 43210. The sulfur regulatory circuit of Neurospora consists of a set of unlinked structural genes which encode sulfur catabolic enzymes and two major regulatory genes which govern their expression. The positive-acting cys-3 regulatory gene is required to turn on the expression of the sulfur-related enzymes, whereas the other regulatory gene, scon, acts in a negative fashion to repress synthesis of the same set of enzymes. The nucleotide sequence of the cys-3 gene was determined and can be translated to yield a protein of molecular weight 25,892 which displays significant homology with the oncogene protein Fos, yeast GCN4 protein, and sea urchin histone H1. The putative cys-3 protein has a well-defined leucine zipper element plus an adjacent charged region which together may comprise a DNA-binding site. A cys-3 mutant and a cys-3 temperature sensitive mutant lead to substitutions of glutamine for basic amino acids within the charged region and thus may alter DNA-binding properties of the cys-3 protein.

N 315 PURIFICATION OF A FACTOR THAT RECOGNIZES THE PHEROMONE RESPONSE ELEMENT (ATGAAACA) OF *SACCHAROMYCES CEREVISIAE*. F. S. Gimble, E. C.-M. Lo, and J. Thorner, University of California, Berkeley, CA 94720. Haploid yeast cells commence the mating process in response to oligopeptide pheromones. An early effect of pheromone treatment is rapid induction of the expression of a number of genes, most of which are involved in mating. Repeats of an eight base-pair consensus sequence, ATGAAACA, are found upstream of all known pheromone-inducible genes. Deletion of these pheromone response elements (PREs) abolishes pheromone-regulated expression in several genes tested. PREs are sufficient for pheromone induction of transcription because insertion of synthetic oligonucleotides containing PREs upstream of the promoter of a *CYC1:lacZ* gene fusion places β-galactosidase expression under hormonal control (10-20X induction). Cell-free extracts fractionated by chromatography on heparin agarose, Affi-gel blue, and an oligonucleotide affinity matrix were assayed for DNA binding activity using PRE-containing probes. In gel retention assays, a single activity was detected which bound to PRE-containing probes, but not to non-specific DNAs. Formation of this DNA-protein complex was inhibited competitively by addition of excess unlabelled oligomers containing the PRE sequence. The same DNA binding activity also "footprints" the PRE sequences within a larger DNA fragment in DNAse I protection assays. This DNA binding activity is present in both induced and uninduced cells, and in both *ste*12 and *ste*5 mutants, suggesting that this activity may be a constitutively expressed transcription factor that is post-translationally activated in response to pheromone. Supported by Damon Runyon-Walter Winchell Cancer Research Fund Postdoctoral Fellowship DRG-909 to F.S.G. and by NIH Research Grant GM21841 to J.T.

N 316 CIS- AND TRANS-ACTING ELEMENTS INVOLVED IN RIBOSOMAL PROTEIN GENE EXPRESSION IN YEAST

Martien H. Herruer, Willem H. Mager, Peter Wessels, Kick C.T. Maurer, Gertrude M. Wassenaar and Rudi J. Planta. Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands.

Upstream of most yeast ribosomal protein genes conserved nucleotide elements occur, designated RPG-boxes, with the consensus sequence AACACCCATACATTT. A synthetic RPG-box can serve as an upstream activation site (UAS) of an heterologous gene. A methylation interference assay revealed the interaction of a protein factor called TUF with the entire element. On the other hand, mutational analyses indicated that only the C at position 6 was indispensible for transcription activation *in vivo*. TUF turned out to be a ubiquitous yeast protein, the cellular amount of which is regulated in a growth-rate dependent fashion. TUF-RPG box complex formation mediates the characteristic nutritional upshift (ethanol → glucose) response on ribosomal protein gene transcription. The 5'-flanking sequence of the S33-gene lacks RPG-boxes. Band shift assays however did reveal the binding of several protein factors. One of these, provisionally called SUF, again is an abundant factor. Methylation interference analysis revealed a dual binding motif: GCCGTCTAGAGTGAC located at -160. Deletion studies demonstrated that SUF-binding is indispensable for efficient transcription activation in yeast cells grown on ethanol. Under glucose conditions however deletion of the SUF-binding site hardly affects transcription activity. Rather, an additional factor binding more downstream is likely to play an important role in S33-gene transcription in glucose-grown cells.

N 317 ANAEROBIC CONTROL OF *COX5b* EXPRESSION, Martin R. Hodge, Kavita Singh and Michael G. Cumsky, Dept. of Molecular Biology & Biochemistry, UC Irvine, Irvine, CA 92717.

In *Saccharomyces cerevisiae* there are two isologous forms of cytochrome *c* oxidase subunit V. Although these subunits are functionally interchangeable, their corresponding structural genes, *COX5a* and *COX5b*, are inversely regulated by oxygen and heme. *COX5a* displays the expected regulatory pattern for a respiratory gene. It is expressed abundantly during aerobic growth, and repressed during anaerobic conditions. In contrast, *COX5b* is expressed predominantly during anaerobiosis. This regulation is mediated by levels of intracellular heme, whose biosynthesis requires oxygen.

We have taken three approaches to elucidate the molecular mechanisms responsible for the anaerobic expression of *COX5b*. In the first, a library of deletion mutants was generated upstream of the *COX5b* coding region. This analysis revealed two elements important for the anaerobic expression pattern. One element appears to be responsible for the aerobic repression of *COX5b* transcription (a URS or Upstream Repression Element). The second is required for efficient anaerobic expression. In our second approach, we are using a gel binding assay to identify the polypeptide factors which interact with these *cis*-acting elements. In the final approach we have selected *trans*-acting mutants which constitutively express the *COX5b* gene.

N 318 COORDINATE TRANSCRIPTION OF NUCLEAR AND MITOCHONDRIAL rDNA SEQUENCES IN THE YEAST S. CEREVISIAE, Alan P. Hudson, Robin Cantwell, and Catherine M. McEntee, Veterans Administration Medical Research Service and Dept. Microbiology and Immunology, The Medical College of Pennsylvania, Philadelphia, PA, 19129

In order to elucidate mechanisms governing rDNA transcription in yeast, we examined the nuclear-mitochondrial (mt) stringent and nutritional upshift responses. In isonuclear ρ^+, 21S rRNA-containing ρ^-, and ρ^0 strains, transcription of nuclear rDNA sequences falls congruently to 15% of control values within 30 minutes after nutritional shiftdown, as assayed via whole cell RNA or by hybrid selection of double labeled 18S+26S transcripts; both in vivo and in an in vitro assay system, the mt stringent response is identical between the ρ^+ and ρ^- strains, and in both the kinetics and magnitude of the mt response mirror those of the nuclear response. When actively growing ρ^+ cells are upshifted from ethanol- to galactose- or glucose-based medium, transcription of nuclear and mt rDNA sequences is congruently increased about two-fold within 30 minutes, as shown by hybrid selection and slot blotting studies. The data suggest that nuclear and mt rDNA transcription are coordinately regulated under some circumstances. These results and others not given here further indicate that the controlling mechanism does not depend on mt transcription or protein synthesis, but rather on some nucleus-encoded, cytoplasmically-synthesized factor(s). Using an in vitro mt transcription assay system, we are now isolating and characterizing the factor(s) responsible for these coordinate nuclear-mt transcriptional responses.
(Supported by the Veterans Administration Medical Research Service)

N 319 GLUCOSE REPRESSION AND THE REGULATION OF GLYCOGEN PHOSPHORYLASE IN *S. CEREVISIAE*, Peter K. Hwang, Stuart Tugendreich, and Robert J. Fletterick, Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143. The *GPH1* gene of *S. cerevisiae* encodes the enzyme glycogen phosphorylase which catalyzes the degradation of glycogen to glucose 1-phosphate, thus enabling yeast to utilize a principal source of reserve energy. *GPH1* transcription appears to be induced during the late exponential phase of growth. A fused *gph1-lacZ* gene was constructed for monitoring the expression of phosphorylase. To examine whether glucose repression is important in regulating phosphorylase expression, yeast cells harboring this construct were grown to exponential phase and then switched into either repressing (2% glucose) or derepressing (0.1% glucose) medium. A ten- to twenty-fold higher level of ß-galactosidase activity was observed in cells incubated in the derepressing medium. In another experiment, cells were grown in medium containing 0.5%, 2%, or 6% glucose. Regardless of which initial glucose concentration was used, expression of *gph1-lacZ* increased only during late exponential growth and reached near maximal levels by the time the medium glucose was half-exhausted. The latter result indicates that *GPH1* is not solely regulated by glucose repression. The effects on *GPH1* expression of the mutations *snf1*, *ssn6*, and *hxk2* that are known to affect glucose repression are under investigation.

N 320 COORDINATION OF YEAST NUCLEAR AND MITOCHONDRIAL TRANSCRIPTION Judith A. Jaehning, Gregory T. Marczynski, Steven E. Wilcoxen, Melinda Tubergan, Mark Parthun, Christopher Stipp and Sei-Heon Jang, Department of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405

The yeast mitochondrial RNA polymerase is composed of a catalytic core and a specificity factor which is involved in recognition of a nonanucleotide promoter. Both components of the enzyme are nuclear encoded. When yeast are grown on a non-repressing carbon source the core component is present at five fold higher levels than when cells are grown on glucose. This increase correlates with an increase in mitochondrial transcription under derepressing conditions. It is possible that regulation of mitochondrial transcription is a simple pathway involving glucose regulated expression of a single nuclear gene. We are altering the regulation and gene dosage of the catalytic core subunit and measuring the effect on mitochondrial RNA synthesis. The nonanucleotide mitochondrial promoter sequence (ATATAAGTA) is found in many nuclear yeast genes including the coordinately regulated GAL7+10, and MEL1 genes. These nuclear sequences function as promoters for the mitochondrial RNA polymerase in vitro and, these same sequences appear to function as "TATA" box promoter elements for RNA polymerase II in the nucleus. Synthetic promoter constucts containing the mitochondrial promoter consensus respond to changes in mitochondrial genotype and are glucose repressed. We have ruled out the possibility that this sequence directs transcription by a form of mitochondrial RNA polymerase present in the nucleus but we are still testing the possibility that a DNA sequence recognition factor may be shared between the two cellular compartments.

N 321 MOLECULAR ANALYSIS OF nuc-1; A GENE CONTROLLING THE EXPRESSION OF PHOSPHATE-METABOLIZING ENZYMES IN *NEUROSPORA CRASSA*

S. Kang and R.L. Metzenberg,Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, WI 53706

A number of phosphate-metabolizing enzymes in *Neurospora crassa* are highly derepressed when the cells are growing in low phosphate medium. The product of the nuc-1^+ gene is required to turn on transcription of the genes for these enzymes. The regulatory system, as revealed by genetic and metabolic analyses, consists of four genes: nuc-2^+, preg$^+$, pgov$^+$, and nuc-1^+. The nuc-1 gene was cloned by chromosome walking and sequenced. Two transcripts, 3.1kb and 2.9kb, are expressed independent of phosphate concentration, implying that the control of the nuc-1^+ activity by negative factor(s) encoded by the preg$^+$ gene and/or the pgov$^+$ gene is at the posttranscriptional level. Sequence analyses of several constitutive mutants(nuc-1^C) and deletion analysis of a wild type (nuc-1^+) gene revealed a domain of the nuc-1^+ protein responsible for this control . Preliminary experiments indicate that the activity of negative factor(s) is controlled in two ways: inhibition by nuc-2^+ gene product and activation by some unknown mechanism, both of which are in turn controlled by phosphate concentration. Sequence analyses of null mutants(nuc-1^-) suggest that the C-terminus of the nuc-1^+ protein is necessary for DNA binding.

N 322 SHORT DNA SEQUENCES FROM THE HO PROMOTER CAN SERVE AS SWI1 DEPENDENT UPSTREAM ACTIVATOR SEQUENCES, Don J. Katcoff, Dept. of Life Sciences, Bar Ilan University, Ramat Gan, Israel.

The controlled transcription of the gene coding for the HO endonuclease in *Saccaromyces cerevisiae* is dependent on at least 10 positive transcription factors called SWI1-10. Mutants in any of these genes result in an absence of HO transcription. Experiments in which upstream sequences from HO were ligated to a heterologous promoter and tested for transcription indicated that SWI1 interacts with at least two different sites upstream of the HO coding region, one found in a fragment about 600 bp long containing URS1, and another in a fragment about 460 bp long containing URS2. Here evidence is presented that SWI1 acts on a short ≤32 bp sequence in URS1, and on another sequence ≤27 bp long in URS2 which bear some similarity to each other. Plasmids were constructed in which these sequences were fused to a partially deleted and inactive CYC1-lacZ reporter gene. ß-galactosidase assays showed that the sequences could serve as UASs when the construct was transformed into yeast. Multiple tandem copies of the sequences in the plasmid enhanced the activity. In swi1 mutants, however, only very low levels of ß-galactosidase were detected regardless of the oligonucleotide copy number. These data support the notion that SWI1 acts via the sequences tested in the HO promoter to promote transcription of HO.

N 323 MECHANISMS OF TRANSCRIPTIONAL REGULATION OF A DNA DAMAGE RESPONSIVE GENE IN *SACCHAROMYCES CEREVISIAE*, Naoko Kobayashi, Terrill McClanahan and Kevin McEntee, Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024

The DNA damage responsive genes of yeast were originally identified by differential hybridization and show increased levels of transcripts following exposure of cells to any of a variety of chemical or physical DNA damaging agents. Two DDR genes, DDRA2 and DDR48 are also induced by heat shock treatment. In addition, the polyubiquitin gene, UBI4 displays regulation by both heat shock and DNA damage. Upstream regulatory region of DDRA2 has been analyzed using a series of BAL31 exonuclease-produced deletions. The results suggest that a region close to the transcription start sites contains binding sites of one or more proteins which negatively controls expression of the gene. These factors can be titrated by increasing the number of copies of their binding sites. Furthermore, the titration results suggest that DDR48 and UBI4 contain common regulatory elements. A second region located between -188 and -142 bp from translational start contains a DNA sequence necessary and sufficient for induction of DDRA2 transcription by either DNA damage or heat shock. This region does not share homology with the well known heat shock consensus element which is crucial for regulation of other heat shock genes.

N 324 GENETIC ANALYSIS OF RAP1, A PUTATIVE GLOBAL TRANSCRIPTIONAL REGULATORY PROTEIN Stephen Kurtz and David Shore, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

The DNA binding protein RAP1 (Repressor/Activator binding site Protein) from S.cerevisiae binds in vitro to a specific sequence found in the regulatory region of the silent mating-type genes and in the upstream regions of many other genes encoding ribosomal proteins, components of the translational system, glycolytic enzymes, RNA polymerase subunits and the MATα regulatory proteins*. Deletions of the RAP1 binding site result in loss of transcriptional repression or activation in a manner dependent upon the context of its binding site. Since the RAP1 gene is essential, we have obtained a set of temperature-sensitive conditional mutations in RAP1 to define the precise roles of RAP1 in transcription. These mutations were generated by mutagenesis of the cloned gene in vitro and were introduced into the cell by a plasmid shuffling technique. Subsequently, the wild-type RAP1 locus has been replaced with the temperature-sensitive alleles. In preliminary analyses, we have observed that all of the mutant strains exhibit a slow death at the non-permissive temperature. Some mutants show a loss of DNA binding activity after several hours growth at the non-permissive temperature. Further characterization will be presented.

*Shore, DM & Nasmyth, KN (1987) Cell 51,721
Buchman, AR et al, (1988) Mol. Cell Biol. 8,210

N 325 STRUCTURE AND FUNCTION OF THE PHO80 GENE OF YEAST, Stephen L. Madden and Lawrence W. Bergman, Department of Chemistry, Clippinger Laboratories, Ohio University, Athens, OH 45701

Acid phosphatase expression from the PHO5 gene is transcriptionally regulated in response to the level of inorganic phosphate in the medium via the interaction of several trans-acting factors. PHO80 is a negative regulatory molecule in this system and is required to repress PHO5 transcription under high phosphate growth conditions. To study the mechanism by which PHO80 represses transcription, we have undertaken the isolation of pho80 mutants using several different mutagenesis schemes. The molecular nature of these pho80 mutants will be discussed. In addition, an assay for the function of the PHO80 molecule is being developed to study the interaction of both wild type and mutant PHO80 molecule with the other factors involved in the expression of acid phosphatase.

N 326 ISOLATION AND CHARACTERIZATION OF AN ASPERGILLUS NIDULANS GENE REQUIRED FOR CONIDIUM PIGMENTATION. Maria E. Mayorga and William E. Timberlake, Departments of Genetics and Plant Pathology, University of Georgia, Athens, GA 30602.

Two Aspergillus nidulans genes, wA and yA, are involved in the production and/or deposition of the mature green pigment of the conidial wall. yA mutants produce yellow spores whereas wA mutants produce white spores. Mutations in the wA gene are epistatic to yA mutations in that spores are white in a $wA^{-}yA^{+}$ strain. However, these strains accumulate the spore specific yA product. The yA gene has been cloned; its transcript accumulates in sporulating cultures but not in vegetative hyphae or in mature conidia (O'Hara and Timberlake, 1989, Genetics, in press). We have cloned the wA gene by complementation of a white-spored mutant strain (yA2,pabaA1;wA3;trpC801) by using an A. nidulans genomic DNA library (Yelton, et al.,1985, PNAS 82: 834). A single yellow transformant was obtained and a cosmid containing the complementing activity was recovered. We have localized this complementing activity to an 8.5 kb SalI-EcoRI fragment. We probed RNA blots of vegetative and developmental cultures with this fragment. The probe hybridized with a ~6.5 kb transcript that is developmentally regulated. We are further characterizing the wA gene and future work will investigate its regulation during development and characterize its role in A. nidulans conidium maturation.

N 327 ISOLATION AND CHARACTERIZATION OF GENES EXPRESSED UNDER MATING CONDITIONS IN Neurospora crassa, Mary Anne Nelson and Robert L. Metzenberg, Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706. Neurospora crassa undergoes a complex pattern of sexual differentiation to form the female reproductive structure (protoperithecium) when subjected to conditions of nitrogen starvation, light and low temperature (25°C or lower). We have isolated genes that are expressed under mating conditions and not during vegetative growth by probing a genomic cosmid library with labelled cDNAs enriched for differentially expressed genes by subtraction hybridization. The clones were mapped to six of the seven Neurospora chromosomes using restriction fragment length polymorphisms; no clustering was noted, although some cosmids appear to encode more than one differentially expressed gene. Many genes expressed only under mating conditions were identified, and the expression of some of these genes was shown to require a functional mating type gene. The expression of these genes under various growth conditions and in mutant strains will be discussed. Also, the results of attempts to inactivate these genes by introduction of multiple copies into recipient strains followed by RIP (rearrangement induced premeiotically)-mediated gene disruptions will be presented.

N 328 CLONING OF AN S.CEREVISIAE GENE THAT IS HOMOLOGOUS IN STRUCTURE, FUNCTION AND TRANSCRIPTIONAL INDUCIBILITY TO THE MAMMALIAN GENE ENCODING HEAVY CHAIN BINDING PROTEIN (BiP). Karl D. Normington, Kenji Kohno, Yasunori Kosutsumi, Mary-Jane Gething and Joe Sambrook. University of Texas Southwestern Medical Center, Dallas, TX. 75235.

The ER of mammalian cells contains a luminal protein of Mr=78Kd (BiP) that binds transiently to prefolded secretory proteins and more permanently to malfolded proteins. Synthesis of BiP mRNA is induced above a constitutive level by reagents as diverse as calcium ionophores, tunicamycin, glucose starvation, and malfolded proteins in the ER. This has led to the hypothesis that the induction of BiP is the result of malfolded proteins in the ER.

We have used a cDNA encoding murine BiP as a hybridization probe to isolate cDNA's encoding a homologous gene of S.cerevisiae. The predicted open reading-frame encodes a 682-amino acid protein that is 63% homologous to murine BiP. Preceding a putative signal peptidase cleavage site is a 42-amino acid sequence capable of translocating the polypeptide into the lumen of the ER. The 4 carboxyterminal residues (HDEL) are reminiscent of the analogous residues of mammalian BiP (KDEL), which may play a role in ER-retention.

Like its mammalian counterpart, yeast BiP is a member of the HSP70 family, eight members of which have been previously recognized in S.cerevisiae. Yeast BiP is a single copy, essential gene in haploid cells. The gene is transcriptionally induced by tunicamycin, glucose starvation and heat shock. *sec* mutants that accumulate proteins in the ER induce BiP mRNA, while a *sec* mutant that blocks translocation causes no induction. Finally, tunicamycin treatment causes nonglycosylated, secreted invertase to coprecipitate with BiP. Taken altogether, yeast BiP is structurally, transcriptionally and functionally homologous to its mammalian counterpart which will allow the further elucidation of BiP's function.

N 329 MALTOSE FERMENTATION IN SACCHAROMYCES CEREVISAE

K.A. Osinga, A.C.H.M. Renniers, J. Welbergen and R.H. Roobol, Research and Development, Royal Gist-brocades N.V., P.O. BOX 1 Delft, The Netherlands.

The first two steps in the fermentation of maltose are the transport of the sugar molecule across the plasma membrane and its hydrolysis to glucose. These processes are catalysed by maltose permease and maltase, respectively. In dough, maltose is formed by the action of α- and β-amylases on the starch of flour. We have investigated whether improvement of maltose uptake and/or conversion to glucose would increase the maltose fermentation and the rate of CO_2 production. To this end, we fused both a maltase and a maltose permease gene to a constitutive promoter.

(Combination of) these genes have been integrated into the genome, both with and without heterologous DNA. The obtained bakers yeast strains show an increased maltose fermentation rate. Application of these strains in dough results in an higher CO_2 production.

N 330 FEEDBACK REGULATION OF HISTONE GENE TRANSCRIPTION IN *S. cerevisiae*,

Mary Ann Osley, Lorraine Moran and Peter Sherwood, Program in Molecular Biology, Sloan-Kettering Cancer Center, New York, NY 10021.

Histones H2A and H2B are encoded by two unlinked genetic loci (*HTA1-HTB1* and *HTA2-HTB2*) in budding yeast. Although a single *HTA-HTB* locus is sufficient for cell viability, numerous phenotypes are associated with strains containing only the *HTA2-HTB2* locus. In contrast, strains with only the *HTA1-HTB1* locus are wild-type. The difference can be accounted for by the ability of the *HTA1-HTB1* locus, but not the *HTA2-HTB2* locus, to derepress its trancription when intracellular levels of H2A and H2B are low. Transcription of the *HTA1-HTB1* locus can also be repressed when extra copies of *HTA* and *HTB* genes are present. Repression is dependent upon overproduction of both H2A and H2B and does not occur when extra copies of single *HTA* or *HTB* genes are present. This regulation is mediated through a negative site in the *HTA1-HTB1* promoter since deletion of this site abolishes repression by H2A and H2B. Mutant strains derepressed for *HTA1-HTB1* transcription in the cell cycle (*hir* mutants) also fail to show repression of this locus by H2A and H2B. These latter observations probably account for the failure of the *HTA2-HTB2* locus to be regulated by intracellular H2A and H2B levels since this locus neither contains a negative site nor is its expression affected by *hir* mutations. We propose that feedback regulation of one *HTA-HTB* locus is used as a mechanism to control histone stoichiometry, a prerequisite for proper nucleosome assembly.

N 331 MOLECULAR CLONING AND REGULATORY ANALYSIS OF THE ARYLSULFATASE STRUCTURAL GENE OF *NEUROSPORA CRASSA*, John V. Paietta, Department of Biochemistry, Wright State University, Dayton, OH 45435. The Neurospora sulfur regulatory system includes a positive regulator, *cys-3*, which controls the synthesis of a number of structural genes (e.g., arylsulfatase, sulfate permease). The *cys-3* protein contains a putative leucine zipper DNA binding element and appears to exert control of the structural genes at a transcriptional level. As part of a study of the sulfur regulatory circuit, the arylsulfatase (*ars*) gene of Neurospora has been cloned. Confirmation of the cloning was obtained by restriction fragment polymorphism analysis and by restoration of arylsulfatase activity through transformation of an arylsulfatase deficient mutant. Northern blot analysis of poly A^+ mRNA showed that arylsulfatase message was present only under derepressing conditions in wild type. In *cys-3* mutants, in which arylsulfatase is not detectable under derepressing conditions, there was a corresponding absence of arylsulfatase message. In the negative regulatory mutant, *scon*^{c}, in which arylsulfatase activity is constitutive, then arylsulfatase message was present under repressing and derepressing conditions. (Supported by NIH grant GM38671).

N 332 TRANSCRIPTIONAL REGULATION OF A YEAST HSP70 GENE, Hay-Oak Park and Elizabeth A. Craig, Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, WI 53706

The SSA1 gene, one of the heat-inducible HSP70 genes in the yeast Saccharomyces cerevisiae, also displays a basal level of expression during logarithmic growth. Multiple sites related to the heat shock element (HSE) consensus sequence are present in the SSA1 promoter region (Slater and Craig, Mol. Cell. Biol. 7:1906, 1987). One of the HSEs, HSE2, is important in the basal expression of SSA1, as well as heat-inducible expression. A promoter containing a mutant HSE2 shows a 5-fold lower level of basal expression and altered kinetics of expression after heat shock. This result implies that the positively acting yeast heat shock factor, HSF, is responsible, at least in part, for the basal level of expression of SSA1. Previous results has suggested that SSA1 is also subject to negative regulation. A series of deletion and point mutations has led to identification of an upstream repression sequence (URS) which overlaps HSE2. A promoter containing a mutation in the URS shows an increased level of basal expression. A URS-binding activity has been detected in yeast whole cell extracts by a gel electrophoresis DNA binding assay. By a series of gel binding competition studies using various oligonucleotides and DNA fragments carrying mutations in HSE2 or the URS, the URS binding protein was distinguished from HSF which binds to the partially overlapping site. The results strongly suggest that the expression of the SSA1 gene is under negative control mediated by a repressor. Currently, we are doing DNase I footprinting to gain better understanding of the mechanism of HSE2-URS regulation.

N 333 **A Protein Involved in Minichromosome Maintenance in Yeast Binds a Transcriptional Enhancer Conserved in Eukaryotes,** Steven Passmore, Randolph Elble and Bik-Kwoon Tye, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853. The *Saccharomyces cerevisiae MCM1* gene product is a protein with multiple functions. It is a transcription factor necessary for expression of mating-type specific genes, and it is required for the maintenance of minichromosomes. MCM1 is a sequence specific DNA binding protein whose activity can be modulated by the α1 protein. It binds to a dyad symmetry element, 5'-C C T A A T T A G G, and related sequences known as MCM1 Control Elements (MCEs). MCEs are found within the regulatory regions *a* and α-specific genes. Direct and indirect DNA binding assays suggest that a conserved ATTAGG in one half of the dyad symmetry element is important for MCM1 binding while variants in the other half are tolerated. These data suggest that MCM1 binds as a dimer, interacting symmetrically with the 5'-ATTAG residues in each half of the binding site. The MCE is similar to the serum response element (SRE) which mediates the response of the human c-*fos* gene to growth factors in serum through the action of the serum response factor. MCM1 binds to the c-*fos* SRE *in vitro* and that, like other MCEs, the c-*fos* SRE exhibits MCM1-mediated UAS activity *in vivo*.

N 334 ISOLATION OF POINT MUTATIONS THAT AFFECT THE LEVEL OF TRANSCRIPTION OF RIBOSOMAL PROTEIN GENE S16A-1 IN Saccharomyces cerevisiae. Sharon M.Papciak and Nancy J. Pearson, Dept. of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228.

Previous work performed in our laboratory has shown that the 5' nontranscribed region of the ribosomal protein gene S16A-1 is sufficient to mediate an increase in the relative rate of synthesis of this protein during a nutritional upshift (1). To further characterize this promoter region we describe here the isolation of point mutations which may be involved in the regulation of S16A-1 during a nutritional upshift. Hydroxylamine was used to mutagenize a plasmid carrying an S16A-1/lacZ fusion, which contains 598 base pairs of promoter sequence upstream from and including a TATA-like box of S16A-1. Restriction fragments carrying the mutagenized promoter region were then purified and ligated to unmutagenized vector DNA. Yeast transformants were screened on X-gal medium containing either glucose or glycerol as a carbon source. Putative mutants were identified as any colony which exhibited a blue color that was either darker or lighter than wild-type colony color. We have isolated four plasmid-associated mutations which reduce the amount of B-galactosidase expression in yeast to 50% or less of wild-type enzyme levels and are in the process of further characterizing these mutations.

(1) Donovan and Pearson, 1986, MCB 6:7, pp. 2429-2435.

N 335 CHARACTERIZATION OF THE PGK GENE OF TRICHODERMA

Sirpa Vanhanen, Anu Jokinen, Päivi Lehtovaara, Merja Penttilä and Jonathan K.C. Knowles, Technical Research Centre of Finland, Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo, Finland

The *pgk* gene coding for phosphoglycerokinase of the filamentous fungus *Trichoderma reesei* was cloned using the corresponding yeast gene as a probe. The gene codes for a protein of 416 amino acids and contains two introns, 219 nt and 75 nt long, at the same positions as the corresponding introns of *Aspergillus* and *Penicillium*. Comparison of the amino acid homologies shows that the PGKs of *Aspergillus* and *Penicillium* are more closely related to each other than to the PGK protein of the imperfect fungus *Trichoderma*. Two transcripts of clearly different size are synthetized from the *Trichoderma* gene. The relative abundance of these mRNAs varies during growth. Expression of the *pgk* gene is being studied in detail.

N 336 GENETIC ANALYSIS OF PLANT TRANSCRIPTIONAL REGULATION USING SCHIZOSACCHAROMYCES POMBE. Celeste Ray, Joy Chien and David W. Ow. Plant Gene Expression Center, USDA/ARS/UC Berkeley, 800 Buchanan Street, Albany, California, 94710.

The study of plant transcriptional regulation has been hampered by the lack of a readily amenable genetic system. To circumvent these difficulties, some groups have examined the activities of plant promoters in the budding yeast Saccharomyces cerevisiae. In these studies, not all plant transcription start sites are used, and novel start sites not seen in plants are apparent. We have explored the use of the fission yeast Schizosaccharomyces pombe as an alternative heterologous system in which to analyze genetically transcription from the CaMV 35S promoter. The firefly luciferase gene was fused to a set of 35S promoter deletions, plasmids containing these fusions introduced into S. pombe, and luciferase activities were determined. We have shown that the pattern of transcription in S. pombe is similar to that seen in carrot protoplasts. Moreover, transcripts of identical length were produced in both plant and yeast cells containing 35S-luciferase fusions. These results, plus the fact that luciferase production by individual yeast colonies can be easily detected, allow us to use S. pombe as a heterologous system to genetically identify the cis and trans-acting factors that regulate transcription from the 35S promoter.

N 337 THE TRANSCRIPTIONAL ACTIVATOR PROTEIN LAC9 INTERACTS WITH THE NEGATIVE REGULATORY PROTEIN GAL80. John M. Salmeron, Jr., Scott D. Langdon, and Stephen A. Johnston. Department of Botany, Duke University, Durham, NC 27706. In Saccharomyces cerevisiae, transcriptional activation mediated by the GAL4 regulatory protein is repressed in the absence of galactose by the binding of the GAL80 protein, an interaction that requires the carboxy-terminal 28 amino acids of GAL4. The homolog of GAL4 from Kluyveromyces lactis, LAC9, activates transcription in S. cerevisiae and is highly similar to GAL4 in its carboxyl terminus, but is not repressed by wild-type levels of GAL80 protein. Here we show that GAL80 does repress LAC9-activated transcription in S. cerevisiae if overproduced. We sought to determine the molecular basis for the difference in the responses of the LAC9 and GAL4 proteins to GAL80. Our results indicate that this difference is due primarily to the fact that under wild-type conditions, the level of LAC9 protein in S. cerevisiae is much higher than that of GAL4, suggesting that LAC9 escapes GAL80-mediated repression by titration of GAL80 protein in vivo. The difference in response to GAL80 is not due to amino acid sequence differences between the LAC9 and GAL4 carboxyl termini. We discuss the implications of these results for the mechanism of galactose metabolism regulation in S. cerevisiae and K. lactis.

N 338 DNA-PROTEIN INTERACTIONS AT THE YEAST SUC2 GENE, Janet Schultz and Marian Carlson, Columbia University, College of Physicians and Surgeons, New York, NY 10032. Expression of the SUC2 (invertase) gene of S. cerevisiae is regulated by glucose repression and the upstream sequence of SUC2 confers glucose-regulated expression to a heterologous gene. We are studying DNA-protein interactions in this regulatory region using gel shift assays. A complex is formed by incubating extract prepared from glucose-repressed cells with a 160 bp fragment from the regulatory region. This complex is not formed if the extract is prepared from glucose-derepressed cells, and instead a number of faster migrating complexes are present. We are examining the effect of mutations in trans-acting factors required for regulation of SUC2 expression on the formation of these complexes. Mutations in the SSN6 gene allow expression of invertase under glucose-repressing conditions, and correspondingly, the complexes formed with extracts from glucose-repressed ssn6 mutants resemble those formed with extracts from derepressed wild type. The product of a bifunctional SSN6-lacZ gene fusion is localized to the nucleus, suggesting that SSN6 plays a direct role in the negative regulation of SUC2. The SSN6 gene product is also necessary for the repression of other glucose-regulated genes and for the repression of a-specific genes in MATα strains. The structure and function of SSN6 is being studied by mutational analysis.

N 339 THE ROLE OF PROMOTER ELEMENTS OF THE 5S rRNA GENE OF SACCHAROMYCES CEREVISIAE IN ASSEMBLY OF A TRANSCRIPTION COMPLEX, Jacqueline Segall and John M. Challice, Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

In vitro transcription of linker-scanning mutations and deletion mutations within the 5S rRNA gene of *Saccharomyces cerevisiae* has revealed 2 essential promoter elements: a control element overlapping the transcription initiation site extends from -5 to +7 and an internal control region (ICR) extends from +81 to +93. Changes in spacing between these two control elements by more than a few base pairs are not tolerated. The ICR is coincident with the site of TFIIIA binding as assessed by methylation interference. The effects of the mutations on the assembly of the 5S gene transcription complex are being assessed. A gel retardation assay is being used to monitor interaction of TFIIIA and TFIIIC with the 5S gene and a template exclusion assay is being used to monitor sequestration of TFIIIB into the 5S gene transcription complex.

N 340 THE ETHANOL REGULON IN *ASPERGILLUS NIDULANS* : IDENTIFICATION OF TARGETS INVOLVED IN THE REGULATION BY *alcR*, THE POSITIVE REGULATORY GENE. Daria Sequeval, Martine Mathieu, Claudio Scazzocchio and Beatrice Felenbok, Institut de Microbiologie, Université Paris -Sud , 91405 Orsay , France.

In *Aspergillus nidulans* the ethanol utilization pathway is under the double control of a positive acting regulatory gene *alcR* which induces the expression of the alcohol and aldehyde dehydrogenases (*alcA* and *aldA*) and of a negative regulatory gene *creA* which mediates carbon catabolite repression.The *alcR* gene is autoregulated, inducible and submitted to carbon catabolite repression like other pathways of *Aspergillus* involved in the utilization of carbon sources (acetamidase, proline).The ethanol regulon has been cloned and entirely sequenced. *alcR* encodes a protein of 96 kD which does not present the known classical structures of DNA-binding proteins. The localization of the sites of interaction between *alcR* encoded protein and the 5' flanking regions of *alcR, aldA* and *alcA* was studied via gel retardation assays and in vitro DNA foot-printing experiments. A putative target for carbon catabolite repression was identified in the 5' flanking region of *alcR* , which is in common with an upstream sequence of *prnB* (the proline permease of the proline utilization pathway). Results of in vitro mutagenesis of this sequence will be discussed.

N 341 IDENTIFICATION OF UPSTREAM FACTORS REGULATING TRANSCRIPTION OF PISATIN DEMETHYLASE, A VIRULENCE GENE IN *NECTRIA HAEMATOCOCCA*, David C. Straney, and Hans D. VanEtten, Department of Plant Pathology, Cornell University, Ithaca New York, 14853.

Nectria haematococca (MP IV) is a pathogen of pea, causing stem and root rot, and produces pisatin demethylase (pda) which detoxifies the phytoalexin pisatin synthesized by the pea. Pisatin demethylase biosynthesis is induced in culture by pisatin and this induction is repressed by glucose. The molecular mechanism by which pisatin induces the pda expression is the target of our investigation.

We have cloned a second pda gene from *N. haematococca* suitable for regulatory studies and have demonstrated that specific mRNA for this gene is induced by pisatin treatment. Using the gel-retention assay we have identified a possible regulatory region upstream from the promoter which binds factors specific to either pisatin induction or glucose repression. The binding region is located within 35 bp. which is 589 bp. upstream from the pda promoter. In vitro binding assays show that pisatin is not a direct effector in causing the pisatin-responsive binding.

N 342 ANALYSIS OF THE PROMOTER OF THE HIGHLY EXPRESSED 3-PHOSPHOGLYCERATE KINASE (PGK) GENE OF Aspergillus nidulans, Stephen J. Streatfield and C.F. Roberts, Department of Genetics, The University, Leicester LE1 7RH, U.K. The gene PGK has been cloned from Aspergillus nidulans and sequenced (Clements & Roberts, 1986, Gene 44, 97-105). Insertion of a selectable marker into the cloned sequence and transformation into a diploid strain of A.nidulans has enabled the location of the PGK gene to chromosome VIII and the recovery of a pgk mutant lacking enzyme activity. The mutant requires both acetate and glycerol for growth and is inhibited by hexoses.

A gene fusion has been constructed to place the lacZ gene of Escherichia coli under the control of the PGK promoter. The A.nidulans catabolic dehydroquinase (QUTE) gene was also incorporated into the vector, and used as a selectable marker for transformation. Transformants were analysed by Southern Blotting to determine the nature of integration events, and were then assayed for β-galactosidase activity to demonstrate the effects of copy number and site of integration on expression of the gene fusion.

Deletions of defined extent have been made into the PGK promoter by exonuclease digestion and these modified constructs transformed into the qutE mutant. Single copy integrants which had been targeted to the resident qutE locus were assayed for β-galactosidase activity to monitor the function of promoter sequences. Particular attention will be focused upon potential CAAT and TATA boxes and a pyrimidine rich sequence located near the transcription start site.

N 343 IDENTIFICATION OF NEW GENES CONTROLLING MATING PHEROMONE-DEPENDENT TRANSCRIPTION IN YEAST, Katsunori Sugimoto, Kunihiro Matsumoto[+], and Roger D. Kornberg, Department of Cell Biology, Stanford University, School of Medicine, Stanford, California 94305, [+]DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304.

The binding of mating pheromone to the yeast cell surface has many consequences that prepare the cell for mating, including arrest in G1 phase of the cell cycle and induction of transcription of a set of genes. One phermone-inducible gene, termed FUS1, is required for cell fusion during the mating process. We have isolated mutants in which a FUS1-lacZ gene is expressed in the absence of pheromone. These mutants fall into four complementation groups. Two of the mutants are recessive and have been denoted cef1 and cef2. Another pheromone-inducible promoter, that of the STE2 gene, was also activated in the absence of pheromone in cef1 and cef2 strains. Effects of these mutations on transcription were mediated by the consensus sequence ATGAAACA. Matches to this consensus sequence are found upstream of all known pheromone-inducible genes and 3 copies of the consensus sequence activated expression of a CYC1-lacZ fusion gene in cef1 and cef2 strains.

N 344 CO-REGULATION OF RIBOSOMAL RNA AND PROTEIN GENES OF NEUROSPORA CRASSA.
Brett M. Tyler, Yuguang Shi and Karin Harrison, Department of Plant Pathology, University of California, Davis, Davis, CA95616.

The synthesis of a balanced supply of components for ribosome assembly requires the co-ordination of three different nuclear RNA polymerases: RNA polymerase I for the large (40S) rRNA precursor, RNA polymerase II for the 70-80 ribosomal proteins, and RNA polymerase III for the 5S rRNA. In order to look for common transcriptional elements with the potential for co-regulating ribosomal genes, we have defined the sequences required for transcription of the 5S rRNA and the 40S rRNA genes, and have cloned and sequenced a Neuropora ribosomal protein gene and its promoter.

We constructed comprehensive sets of deletions and point mutation clusters spanning the promoter regions of 5S and 40S rRNA genes and tested the effects of these mutations using homologous in vitro transcription assays. The results show that transcription of the 5S genes requires a TATA-box at -29, which fixes the startpoint of transcription, plus three internal elements including a novel element, the RIBO box, at +18 to +34 (1).

Transcription of the 40S gene requires two broad domains located at -116 to -36 and -28 to +4, plus a sequence between -240 and -116 which provides a 2-3 fold stimulation. The downstream domain includes a TATA-box at -5 which is absolutely required for transcripton. The upstream domain includes a RIBO box located at -64 strongly homologous to that of the 5S, raising the strong possibility that the RIBO box co-ordinates transcription of the 5S and 40S rRNA genes in Neurospora.

The Neurospora ribosomal protein gene we have sequenced (crp-2) proved to contain three RIBO boxes in its promoter region at -271, -89 and -9, the last with 15/17 matches to the 5S RIBO box. We are currently testing how the RIBO boxes influence the transcription of crp-2 and other genes such as qa-2, and are identifying proteins which bind the RIBO box.

(1) Tyler, BM (1987) J. Mol. Biol. 196, 801-811

N 345 ANALYSIS OF EXPRESSION SIGNALS OF THE ASPERGILLUS NIDULANS gpdA GENE, Punt P.J., Kuijvenhoven J., Dingemanse M.A., Pouwels P.H., van den Hondel C.A.M.J.J., Medical Biological Laboratory, TNO, P.O. BOX 45, 2280 AA Rijsijk, The Netherlands.

Regulation of gene expression in Aspergillus has been extensively investigated by genetic means. However, little is known about gene expression at the molecular level. Recently a limited number of studies was carried out to analyze cis-acting genetic elements that control expression of some Aspergillus genes. In some of these studies an E. coli lacZ (β-galactosidase)-fusion system adapted for Aspergillus was used (van Gorcom et al.,Gene 48, 211-217, 1986).

We are carrying out a detailed analysis of the expression signals of the higly expressed A. nidulans glyceraldehyde-3-phosphate dehydrogenase (gpdA) gene. The main strategy followed is the generation of promoter mutations by either Bal31 digestion of DNA regions of variable length or by deletion of conserved sequences also present in other highly expressed and/or glycolytic Aspergillus genes. The effect of these mutations was studied by fusion of the mutated promoters to the lacZ gene and determination of the lacZ expression levels.
The results of the promoter analysis will be presented and discussed.

N 346 PURIFICATION AND CHARACTERIZATION OF SACCHAROMYCES CEREVISIAE TRANSCRIPTION FACTOR IIIA. C. Kathy Wang, and P. Anthony Weil, Dept. of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232 and Dept. of Biochemistry, University of Iowa, Iowa City, Iowa 52242.
Saccharomyces cerevisiae Transcription factor IIIA (TFIIIA) has been purified to apparent homogeneity. Two polypeptides copurified with TFIIIA activity. Yeast TFIIIA is a DNA-binding protein which exhibits a high affinity for the internal control region of the homologous 5 S ribosomal RNA gene. Characterization of the yeast protein indicates that it shares most, but not all, of the molecular properties of its Xenopus TFIIIA counterpart.

N 347 ISOLATION AND CHARACTERISATION OF HSP70-LIKE GENES FROM *S.POMBE*,
Felicity Z. Watts and Michael J. Powell, School of Biological Sciences, University of Sussex, Falmer, Brighton, East Sussex, U.K.
The genome of *S.pombe* contains a family of genes related to the heat inducible HSP70 genes of other eukaryotes, and to the *E.coli dnaK* gene. We have isolated a number of members of this gene family from cDNA and genomic libraries of *S.pombe*. DNA sequence analysis indicates that these genes have approximately 70% homology to certain heat shock genes in *Drosophila* and *Xenopus*.
We have analysed the expression of these HSP70-like genes before and after heat shock. Two of the genes show increased levels of expression (30 and 200 fold) after heat shock. A third gene shows no altered expression suggesting that it may encode an HSP70 cognate. Sequence data relating to the promoter sequences will be presented.

N 348 IDENTIFICATION OF PROTEIN-DNA INTERACTIONS REQUIRED FOR *GCR1* CONTROL OF *ENO1* AND *ENO2*
Catherine E. Willett, Janice P. Holland, Paul Brindle and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616
In *Saccharomyces cerevisiae*, several genes encoding enzymes of the glycolytic pathway are coordinately regulated by the positive transcriptional regualtory gene *GCR1*. We are concerned specifically with the two enolase genes, *ENO1* and *ENO2* as a model for this regulation. Deletion mutations have been constructed within the UAS regions of both *ENO1* and *ENO2* that render expression of each gene independent of *GCR1*. This suggests that transcription of *ENO1* and *ENO2* is modulated by a negative regulator that interacts with the UAS regions of both genes, and that the *GCR1* gene product acts to antagonize this negative regulator. Footprinting studies have identified a protein binding site in the *ENO2* UAS region indicated by deletion analysis to be important for *GCR1* regulation. Binding of this protein is modulated by *GCR1*, however, the binding protein is not encoded by the *GCR1* gene. Since *GCR1* regulates both *ENO1* and *ENO2* in a similar manner, we expect factors required for *GCR1* control to be shared between the two genes. In the case of *ENO1*, two binding events have been identified by footprinting analyses. Utilizing double-stranded oligonucleotides correspinding to the two binding sites, the proteins responsible for each binding event were identified after ion exchange chromotography of yeast whole cell extracts. The *RAP1* transcription factor binds to one of the sites while the second binding protein is unrelated to either the *RAP1* factor or the factor that binds specifically to the *ENO2* UAS region. Gel shift analysis revealled a third binding event within the *ENO1* 5' region. This latter binding factor corresponds to the *GCR1*-dependent binding protein identified for *ENO2*. It is clear that multiple binding proteins interact with the UAS region of *ENO1* and *ENO2*. We speculate that *GCR1*-dependent control of expression of these genes requires a binding protein that interacts specifically with the UAS regions of both genes and is distinct from the *RAP1* transcription factor.

N 349 SEQUENCE OF MAL64 MUTANT ALLELES ENCODING TRANS-ACTING FACTORS FOR CONSTITUTIVE EXPRESSION OF THE MALTOSE FERMENTATIVE GENES, Lori A. Young, Corinne A. Michels, Departments of Biology and Biochemistry, Queens College and the Graduate School of CUNY, Flushing, NY 11367. The MAL6 locus is a cluster of three genes encoding maltose permease (MAL61 gene), maltase (MAL62 gene) and a trans-acting positive factor required for maltose inducible transcription of MAL61 and MAL62 (MAL63 gene). Maltose fermenting revertants of mal63 non-inducible mutants were isolated and these were found to constitutively express maltase and maltose permease. The alterations in the constitutive revertants mapped to a new, MAL6-linked gene, called MAL64, which is highly sequence homologous to MAL63. Two constitutive alleles of MAL64 have been characterized, MAL64-C2 and MAL64-R10, and these are proposed to encode trans-acting positive factors required for the constitutive expression of MAL61 and MAL62. In inducible strains, the MAL64 product is dispensible. The wild type sequence of MAL64 has been determined and compared to that of its homolog, MAL63. There is extensive sequence homology between the two at both the DNA and amino acid level including the faithful conservation of a cysteine-zinc-finger DNA binding domain. Since the wild type MAL64 gene is not required for the inducible regulation of the maltose fermentative enzymes, sequencing the MAL64 constitutive mutant alleles will allow us to identify the structural change(s) in this protein responsible for its altered function in constitutive strains. Both the MAL64-C2 and MAL64-R10 alleles are cloned and their gene sequences will be presented.

Post-Transcriptional Control Mechanisms and Pathogenesis

N 400 CHARACTERIZATION OF TRICHODERMA REESEI EG I PROTEIN EXPRESSED IN SACCHAROMYCES CEREVISIAE BY DELETION ANALYSIS AND A MONOCLONAL ANTIBODY, Aho, S., Paloheimo, M. and Korhola, M., Research Laboratories, Alko Ltd. POB 350, 00101 Helsinki, Finland

The egl1 gene and cDNA have been cloned from Trichoderma reesei strain VTT-D-80133 (1). We made nine 3'-deletions covering the EG I cDNA using exonuclease III. The full length cDNA and the deletions were cloned under the control of the ADC1-promoter into pAAH5-plasmid and expressed in a laboratory strain Yf135 of Saccharomyces cerevisiae. Native proteins secreted by yeast cells grown on nitrocellulose filters placed on the top of nutrient agar plates were detected by a monoclonal antibody raised against native EG I protein from Trichoderma reesei. Denatured proteins were studied on Western blots using the same monoclonal antibody, after samples from disrupted yeast cells and concentrated culture medium from shake flask cultures were run in SDS-PAGE. EG I enzyme activity secreted from yeast cells expressing the truncated proteins was detected by growing cells on OBR-HEC plates (2). We found that the antigenic epitope for the monoclonal antibody is located in the N-terminal half of the protein. The carboxyterminal end with its O-glycosylated region has been suggested to function as a substrate binding region for the cellobiohydrolase enzyme. It could be removed from the EG I protein and the protein still retained its activity against the soluble substrate but as soon as the region next to it was touched, EG I enzyme activity was lost.

Using these results and those from the experiments in progress we wish to learn more about the functional and structural characteristics of this industrially important enzyme.

1. Penttilä M, Lehtovaara P, Nevalainen H, Bhikhabhai R and Knowles J (1986) Gene 45, 263-263.
2. Farkas V, Liskova M and Biely P (1985) FEMS Microbiol. Letters 28, 137-140.

N 401 MUTAGENESIS OF THE *S. cerevisiae* U2 GENE REVEALS AN ESSENTIAL U2 RNA STRUCTURAL ELEMENT, Manuel Ares, Jr. and A. Haller Igel, Department of Biology, University of California, Santa Cruz, CA 95064. U2 RNA is a member of a class of small nuclear RNAs required for splicing of nuclear premessenger RNA. *Saccharomyces cerevisiae* contains a single essential U2 gene (called *SNR20*, also *LSR1*), which contains a highly conserved region characteristic of U2 RNAs. Models of U2 secondary structure consistent with phylogenetic variation predict two helices with potential to form a pseudoknot, or to participate in two alternative conformations of U2 RNA. Using oligonucleotide mutagenesis and a plasmid shuffling technique, we constructed and tested 19 mutations of a 55 nucleotide region of U2 RNA containing these conserved helices. Single mutations were designed to disrupt the ability of RNA strands to pair, such that appropriately combined double mutations restore pairing. Single mutations that disrupt pairing between positions 50-53 and 62-65 have lethal or cold sensitive lethal phenotypes, whereas double mutations that alter the primary sequence, but preserve the ability to pair are viable. As many as 3 of 8 predicted base pairs in the second helix can be disrupted without affecting U2 function, suggesting that this helix is dispensable, in spite of its conservation in fungi, plants, and animals. Additional mutations have been constructed to confirm this observation, and molecular studies have been undertaken to determine the effect of mutations on the structure of the U2 snRNP. The essential helix is formed by pairing of nucleotides at positions 48-53 with those at positions 62-67, and represents the 3' boundary of the most highly conserved region of U2.

N 402 CHARACTERIZATION OF A KLUYVEROMYCES LACTIS SEC14p, Vytas A. Bankaitis and Sofie Salama, Department of Microbiology, University of Illinois, Urbana, IL 61801.
The S. cerevisiae SEC14p behaves as a cytosolic factor that is essential for yeast Golgi function. Radiolabeling experiments have revealed the presence of SEC14p cross-reactive polypeptides in extracts prepared from the yeasts Kluyveromyces lactis and Schizosaccharomyces pombe. We have obtained a clone of K. lactis genomic DNA that complements the S. cerevisiae sec14-1^{ts} defect. Further analysis has shown that the complementing clone programmed the synthesis of the K. lactis SEC14p antigenic homolog in S. cerevisiae. This K. lactis SEC14p was capable, in single copy, of fully substituting for the S. cerevisiae SEC14p in sec14-1^{ts} and sec14 null mutants with respect to growth, secretion, and vacuolar protein traffic. Nucleotide sequence analysis has shown the S. cerevisiae and K. lactis SEC14p's to exhibit an almost 80% identity at the primary sequence level. These data suggest a conservation of SEC14p structure and function across widely diverged yeast species.

N 403 THE MOLECULAR GENETICS OF BENZIMIDAZOLE RESISTANCE IN PSEUDOCERCOSPORELLA HERPOTRICHOIDES, Emily J. Blakemore, Mark J. Hocart, John A. Lucas and John F. Peberdy, Department of Botany, University of Nottingham, Nottingham NG7 2RD, U.K.
Pseudocercosporella herpotrichoides is the cause of eyespot disease in winter cereals. Until recently the fungus was routinely controlled by benzimidazole fungicides but most field isolates are now resistant to this group of compounds. We are working towards an understanding of the genetic basis of benzimidazole resistance in P. herpotrichoides The primary mode of action of benzimidazole fungicides involves binding to β-tubulin protein and consequent destabilisation of microtubules. Resistance to this group of fungicides has been shown in some species to result from alteration of the β-tubulin.

A genomic library of P. herpotrichoides has been made from which a β-tubulin gene has been isolated. To investigate the expression of this cloned gene a transformation system has been developed. Four selection systems, including resistance to benomyl, methyl (3,5-dichlorophenyl) carbamate and hygromycin B and acetamide utilisation, were used to establish successful transformation in this fungus.

N 404 70K STRESS PROTEINS BIND PRECURSORS OF SECRETORY AND MITOCHONDRIAL PROTEINS, William J. Chirico and Günter Blobel, Laboratory of Cell Biology, The Howard Hughes Medical Institute at The Rockefeller University, 1230 York Avenue, New York, NY 10021.
70K stress proteins were previously shown to facilitate translocation of protein precursors into yeast microsomes and mitochondria in vitro. Similarly, in vivo studies showed that yeast strains deficient in 70K stress proteins accumulate precursors of secretory and mitochondrial proteins in the cytosol. Although 70K proteins may re-fold or maintain precursors in a translocation competent conformation, a direct association between 70K stress proteins and precursors has not been demonstrated. We report here that a presecretory protein (prepro-alpha-factor) or a mitochondrial precursor (beta subunit of F1 ATPase), which were first synthesized in a wheat germ in vitro translation system, can form a stable complex with 70K stress proteins. The complexes were immunoprecipitated with polyclonal antibodies raised against yeast 70K stress proteins. In vitro synthesized preprolactin and prolactin also were immunoprecipitated with these antibodies. In contrast, only about 5% of in vitro synthesized globin, a cytosolic protein, was immunoprecipitated. These data suggest that if a specific structural motif is recognized by 70K stress proteins then the hydrophobic signal sequence does not contain all the binding information. Additional information, therefore, may reside in the pro region of presecretory proteins and in the basic presequence of mitochondrial precursors.

N 405 SELECTION OF TRANSLATION INITIATION SITES IN YEAST, John Clements*, Thomas Laz and Fred Sherman, Departments of Biochemistry and Biophysics, University of Rochester, Rochester, New York 14642. *Present address, British Biotechnology Ltd., Watlington Rd., Oxford OX4 5LY, UK. The structures and sequences affecting the expression and selection of translation initiation sites in yeast were investigated with a fused gene composed of the leader region of the yeast CYC7 gene and coding region of the E.coli lacZ gene. The following major conclusions were reached by measuring β-galactosidase activities in yeast strains having integrated single-copies of the fused genes with various alterations in the untranslated leader region. AUG codon is the only codon which can efficiently initiate translation although some non-AUG codons allow initiation but at low efficiency below 1% of the normal level. The nucleotide preceding the AUG initiator at position -3 modified the efficiency of translation by less than two fold, exhibiting an order of preference A>G>C>T. Upstream out-of-frame AUG triplets diminished initiation at the normal translation initiation site, from essentially complete inhibition to approximately 73% inhibition depending on the context of the two AUG triplets. The introduction of terminator codons in-frame with the upstream AUG codons did not prevent the inhibition of the upstream AUG codon. Thus yeast and higher eukaryotes appear to have a similar process for initiating translation, except that translation in yeast does not reinitiate after a termination codon.

N 406 MUTATIONS IN RSD1 (SAC1) SUPPRESS DEFECTS IN YEAST GOLGI AND YEAST ACTIN FUNCTION, Ann E. Cleves and Vytas A. Bankaitis, Department of Microbiology, University of Illinois, Urbana, IL 61801.

The S. cerevisiae SEC14p appears to play a direct role in yeast Golgi function. Because inspection of the inferred SEC14p primary sequence failed to reveal clues as to biochemical activities that may be associated with SEC14p function, we undertook a pseudoreversion analysis of sec14-1^{ts} to identify gene products that either modulate SEC14p function or are responsive to it. We have isolated 26 recessive and 81 dominant suppressors of sec14-1^{ts}. The recessive suppressors define 4 complementation groups (rsd1-4) while the dominant suppressors fall into two linkage groups (dsd1 and dsd2). Of these mutants, the rsd1 group has been most extensively studied. All 14 members of rsd1 had simultaneously acquired a new cs phenotype, in addition to the selected Ts$^+$ suppressor phenotype. The wild-type RSD1 has been cloned and sequenced, and the null phenotype is cold-sensitivity for growth. However, rsd1 null mutations do not suppress sec14-1^{ts}. We have shown that RSD1 is allelic to SAC1, a gene identified by Novick and Botstein as a highly allele-specific suppressor of yeast actin defects. These data suggest a relationship between SEC14p and actin in the yeast secretory pathway.

N 407 **NUCLEOTIDES REQUIRED FOR 3' SPLICE SITE RECOGNITION IN THE ACTIN INTRON OF THE YEAST *KLUYVEROMYCES LACTIS*,** Deshler, J.D. and Rossi, J.J., Department of Microbiology, University of California, Los Angeles, CA 90024 and Beckman Research Institute, City of Hope, Duarte, CA 91010.

We have previously shown that the budding yeast *Kluyveromyces lactis* utilizes identical intron consensus sequences as *Saccharomyces cerevisiae*. Subsequently, we demonstrated splicing of heterologous *ACT* transcripts in *S. cerevisiae* and *K. lactis*. This result suggests that nonconserved sequence elements required for splicing are reciprocally functional between these two organisms. In short, intron recognition and processing signals for all practical purposes appear identical between *K. lactis* and *S. cerevisiae*.

Here, we have focused on a curious feature of the *K. lactis ACT* intron. The 3' splice site is located 125 nucleotides from the TACTAAC branching sequence. Within this unusually long spacing, resides a PyAG which is 71 nucleotides from the branch point. The obvious questions to us were, why is the first PyAG skipped, and how is this unusually long spacing, which normally inhibits the second step of splicing, structured such that the appropriate PyAG is recognized as the 3' splice site? We have used *K. lactis ACT-E. coli lacZ* fusions to help answer these questions. The region between the TACTAAC and 3' splice site was randomly mutagenized, and colonies where picked which harbored aberrantly spliced *ACT* RNAs. Mutations which affect the 3' splice site selection in this gene will be presented and their implications, discussed.

N 408 ROLE OF THE REGION AROUND THREONINE ^{1651} IN THE REGULATION OF ADENYLATE CYCLASE ACTIVITY IN THE YEAST *SACCHAROMYCES CEREVISIAE*: A MUTATIONAL ANALYSIS. G. Feger, E. De Vendittis, A. Vitelli, R. Zahn and O. Fasano, Differentiation Programme, European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG.

We have previously described the isolation of a spontaneous chromosomal suppressor of the temperature-sensitive yeast strain TS1 (1). Molecular analysis revealed a point mutation in the adenylate cyclase gene, replacing a threonine by a isoleucine at position 1651. Biochemically the mutant adenylate cyclase showed a constitutive activation as well as a hypersensitivity to the *RAS* gene products *in vivo* and *in vitro*. The amino acid sequence surrounding threonine 1651 fits a consensus sequence for protein kinase C. This suggests the regulation of the *RAS*-dependent adenylate cyclase activity by cellular protein kinases. To investigate this possibility we performed site-directed mutagenesis of the adenylate cyclase gene in the region of interest.
Analysis of the so generated mutant adenylate cyclase alleles in respect to their response to different *RAS2* alleles and their implication for the regulation of adenylate cyclase by protein kinases will be discussed in detail.

(1) De Vendittis, E. et al., EMBO J. 5, 3657-3663 (1986)

N 409 DEPLETION OF AN ESSENTIAL snRNA DISRUPTS 18S RIBOSOMAL RNA SYNTHESIS IN *SACCHAROMYCES CEREVISIAE*. Maurille J. Fournier and Haodong Li, Department of Biochemistry, University of Massachusetts, Amherst, MA 010003.
At least nine *Saccharomyces cerevisiae* small nuclear RNAs (snRNAs) have been implicated in ribosome biogenesis, from nucleolar localization data and demonstrated associations with pre-rRNA species (D.Tollervey, EMBO J. *6*:4169-75, 1987; J. Zagorski *et al*, Mol. Cell. Biol. *8*:3282-90, 1988). Disruption of a gene for one of these snRNAs, the non-essential *SNR10*, was shown to slow processing of 35S pre-rRNA (EMBO J, *ibid*). In the current study, we have examined the effect of depleting the cell of one of the essential snRNAs associated with ribosome synthesis. The RNA of interest contains 128 bases and is designated snR128 (Mol. Cell. Biol., *ibid*). Our strategy involved repression of snR128 synthesis in cells dependent on a *GAL1::SNR128* allele.
Repression was shown to result in impaired production of mature 18S rRNA. The effect was evident within one generation after the onset of *SNR128* gene repression and correlated well with depletion of the recombinant snRNA. The steady state mass ratio of 18S:25S RNA eventually decreased about eight-fold. Results from pulse-chase assays revealed a block in processing of 35S pre-rRNA, apparently at the sites that define the 20S intermediate that contains 18S RNA. The results constitute the first demonstrated involvement of an essential snRNA in rRNA maturation.

N 410 EFFECT OF MUTATIONS WITHIN LUCIFERASE, A PEROXISOMAL PROTEIN, ON ITS SUBCELLULAR DISTRIBUTION IN YEAST CELLS, Stephen J. Gould, Gilbert-Andre Keller, and Suresh Subramani, Department of Biology, University of California, San Diego, La Jolla, CA 92093. Firefly luciferase is targeted to peroxisomes in both insect and mammalian cells. Furthermore, transport of the protein has been shown to be mediated by a three-amino-acid-long COOH-terminal targeting signal.
To determine whether firefly luciferase could be transported into peroxisomes in yeasts as well as in higher eukaryotes, we introduced the cDNA encoding luciferase into the yeasts Saccharomyces cerevisiae and Hansenula polymorpha. Immunocryoelectron microscopic analysis of yeast cells expressing the heterologous protein demonstrated that luciferase was transported to peroxisomes in both of these yeasts. It is reasonable to postulate that the peroxisomal targeting signal that directs luciferase to peroxisomes in yeast cells is the same as the one which directs it to peroxisomes in mammalian cells. To directly test whether this is true, we have expressed a number of mutant luciferases (previously used to define the peroxisomal targeting signal in luciferase that is active in mammalian cells) in Saccharomyces cerevisiae. The subcellular distribution of these mutant luciferase proteins within the yeast cell will be presented and will allow for a comparison of the peroxisomal targeting signal active in yeast and that which is active in mammalian cells.

N 411 THE ACETYL-COENZYME A SYNTHASE GENE AS A HOMOLOGOUS SELECTABLE MARKER FOR TRANSFORMATION OF THE CORN SMUT PATHOGEN, *USTILAGO MAYDIS*. John.A.Hargreaves [1] and Geoffrey Turner [2],1.)Molecular Pathology Group, Long Ashton Research Station, Institute of Arable Crops Research, Long Ashton, Bristol, BS18 9AF, U.K ,2.) Department of Microbiology, University of Bristol,Bristol, BS8 1TD, U.K. The acetyl-Co A synthase (*Acu*) gene of *U.maydis* has been isolated and used to transform *Acu*⁻ mutants of *U.maydis* to acetate utilization. The *Acu*⁻ mutants were recovered by positive selection for resistance to fluoroacetic acid. The *U.maydis Acu* gene was isolated using a fragment of the coding region of the equivalent gene (*Acu5*) from *Neurospora crassa* as a heterologous probe. In southern blots of *U.maydis* genomic DNA digested with a number of restriction enzymes, a *Hind* III digest showed hybridization of the probe to a single fragment (c.7kb) at a stringency allowing approximately 75% homology. This fragment was isolated from a restriction fragment enriched plasmid bank and used to transform four separate *Acu*⁻ mutants, to growth on acetate, at a frequency of 5 - 20 transformants per ug DNA. Southern analysis of selected transformants showed that transformation was accomplished by integration of vector sequences into the choromosomal DNA. In most cases, the hybridization signals were consistent with the integration of a single copy of the vector. In one case, the transforming wild type gene appeared to have replaced the mutant host gene.

N 412 RNases INVOLVED IN THE PROCESSING OF MITOCHONDRIAL RNA's OF YEAST, Ted J. Hofmann, and H. Peter Zassenhaus, Department of Microbiology, St Louis University School of Medicine, St. Louis, Mo. 63104. Mitochondrial RNA's in Saccharomyces cerevisiae are first synthesized as long polycistronic transcripts which subsequently undergo a variety of site-specific processing events; yet the enzymes which regulate these events have not been previously described. We will report on the characterization of three enzymes which specifically act on RNA: (1) an endonuclease which cleaves preferentially 5' to pyrimidine-adenine dinucleotides, (2) an exonuclease dependent on nucleotide triphosphates for activity, and (3) and RNaseH activity which co-purifies with a complex including the DNA polymerase. The function of these enzymes in mitochondrial nucleic acid metabolism is being probed by the isolation of mutants deficient in their activity. We will report on two mutants which were isolated as nuclear suppressors of mitochondrial mutants affecting RNA processing.

N 413 USE OF CHIMERIC mRNAS TO STUDY THE STRUCTURAL DETERMINANTS OF mRNA STABILITY IN THE YEAST *SACCHAROMYCES CEREVISIAE*, Allan Jacobson, David Herrick, and Roy Parker, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655.
As an approach to understanding the structural determinants of mRNA stability in yeast we have measured the decay rates of 21 mRNAs encoded by well-characterized genes and constructed approximately 30 different chimeric genes in which we have either: a) exchanged 5' and 3' halves of molecules, b) exchanged 3' regions exclusively, or c) deleted specific regions. Decay rates of the "parental" and chimeric mRNAs were measured by RNase protection or northern blotting assays using RNA that had been extracted from a temperature-sensitive RNA polymerase II mutant (Nonet *et al*, **MCB** 7:1602-1611, 1987) at different times after a shift from 24°C to 36°C. Our objective was to determine whether the 25-30' half-life of a stable mRNA (e.g., **ACT1** or **PGK1**) could be reduced to the 3-5' half-life of unstable mRNAs (e.g., **STE2**, **STE3**, **HIS3**, or **MFα1**) by dominant, cis-acting "instability elements." Our results are consistent with the existence of such elements within: a) the 5'-UT of **STE3**, b) the 5' half of **HIS3**, and c) the 3' half of **MFα1**. The introduction of nonsense codons into chimeric mRNAs containing the **MFα1** element demonstrates that expression of the instability phenotype associated with the **MFα1** element is dependent on ribosomal translocation up to or through the element. Experiments in progress are designed to delineate the **HIS3** and **MFα1** elements more precisely and to identify the essential nucleotides in the **STE3** element.

N 414 YEAST PHASE SPECIFIC GENE EXPRESSION IN THERMOTOLERANT AND THERMOSENSITIVE STRAINS OF HISTOPLASMA CAPSULATUM (Hc), Elizabeth J. Keath, Eric D. Spitzer, Audrey A. Painter, Sharon J. Travis, Judith Medoff, George S. Kobayashi, and Gerald Medoff. Division of Infectious Disease, Washington University School of Medicine, St. Louis, MO 63110.

A 1.9 kb Hind III probe from a Histoplasma-specific nuclear gene, yps-3, hybridizes to three poly A+ RNAs pf 1.3, 1.1 and 0.95 kb from the yeast (Y) phase of the thermotolerant strain G217B. These mRNAs are absent in poly A+ RNA isolated from mycelia (M). This high virulence strain can undergo the M-Y phase transition at 40°C, and the yeast phase can grow at this temperature. In contrast, the thermosensitive strain, DOWNS, fails to express the yps-3 gene in either M or Y phases, although southern analysis confirms the presence of the yps-3 homolog. DOWNS is a low virulence strain which cannot transform to or grow as a yeast at temperatures exceeding 37°C. Clinical isolates of Hc obtained from patients with AIDS exhibited the DOWNS phenotype, while Hc strains from non-AIDS patients with disseminated disease expressed the yps-3 gene in the Y phase and had the G217B phenotype. The epidemiology of the DOWNS strain and the correlation between yps-3 expression and the more complex phenotypes of thermotolerance and virulence are under investigation.

N 415 MOLECULAR CLONING AND CHARACTERIZATION OF THE *AAA1* GENE ENCODING N^{α}-ACETYLTRANSFERASE FROM *SACCHAROMYCES CEREVISIAE* Fang-Jen S. Lee, Lee-Wen Lin and John A. Smith, Departments of Molecular Biology, and Pathology, Massachusetts General Hospital, and Departments of Genetics and Pathology, Harvard Medical School, Boston, MA 02114.

Acetylation is the most frequently occurring chemical modification of the α-NH_2 group of eukaryotic proteins and is catalyzed by a N^{α}-acetyltransferase. Recently, a eukaryotic N^{α}-acetyltransferase was purified to homogeneity from *Saccharomyces cerevisiae*, and its substrate specificity was partially characterized (Lee, F-J. S., Lin L-W., and Smith, J.A., J. Biol. Chem. **263**:14948-14955, 1988). A full-length cDNA encoding yeast N^{α}-acetyltransferase was cloned from a yeast λgt11 cDNA library and characterized (Lee, F-J. S., Lin L-W., and Smith, J.A., Proc. Natl. Acad. Sci. USA, in press, 1989). The yeast N^{α}-acetyltransferase protein is encoded by an open reading frame of 2562 bases. The molecular weight calculated from the amino acid composition is 98,575 daltons, which agrees well with the previously determined subunit M_r of 95,000 ± 2,000. DNA blot hybridizations of genomic and chromosomal DNA reveal that the gene (so-called *AAA1*, *a*mino-terminal, α-*a*mino, *a*cetyltransferase) is present as a single copy located on chromosome IV and is positioned immediately upstream of the *SIR2* gene. The null mutation (*aaa1*-Δ) by gene replacement, while not lethal, makes cells grow slowly. The use of this cDNA will allow the molecular details of the role of N^{α}-acetylation in the sorting and degradation of eukaryotic proteins to be determined.

N 416 INFLUENCE OF THE GENETIC CONTEXT OF SACCHAROMYCES CEREVISIAE HOST STRAINS ON HIRUDIN PRODUCTION, Yves Lemoine, Martine Nguyen-Juilleret, Muriel Merkamm, Carolyn Roitsch, and Tilman Achstetter, Transgene S.A., F-67000 Strasbourg, France

Hirudin is a potent anticoagulant that is secreted from the salivary gland of the leech Hirudo medicinalis. It forms a stable complex with thrombin in a 1:1 molar ratio completely blocking the proteolytic activity of the enzyme. Natural hirudin is composed of a family of closely related variants of 65-66 amino acids all of them having a cystein-rich N-terminal domain and an acidic C-terminal region. The cDNA encoding variant 2 (rHV2) has been used to produce hirudin in S. cerevisiae (1,2). It has been fused in frame to sequences coding for secretion signals of the alpha-factor pheromone precursor from yeast allowing the export into the culture supernatant (2). Specific and non-specific modifications in the host's genetic background will be described that influence integrity and the level of productivity of recombinant hirudin. The pharmaceutical development of recombinant hirudin is being pursued within a partnership agreement between Transgene, 16, rue Henri Regnault, 92411 Courbevoie Cedex, France and Sanofi, 40, Avenue Georges V, 750008 Paris, France.

(1) Harvey, R.P. et al. (1986) PNAS 83, 1084-1088
(2) Loison, G. et al. (1988) Bio/Technology 6, 72-77

N 417 IN VITRO STUDIES OF YEAST mRNA SPLICING USING TEMPERATURE-SENSITIVE rna MUTANTS, R.-J. Lin, Department of Microbiology, University of Texas, Austin, TX 78712.
We have shown that a whole-cell yeast extract accurately splices synthetic pre-mRNA of yeast nuclear genes in vitro, and the splicing takes place on a 40S particle called the spliceosome. This in vitro mRNA splicing system has been used to study temperature-sensitive mutants (rna2-11) which accumulate intron-containing pre-mRNA at the restrictive temperature. We have found that extracts prepared from most of the rna mutants are heat sensitive for mRNA splicing activity. Furthermore, most of the heat-inactivated mutant extracts, with the exception of rna2, do not form the spliceosome, suggesting their gene products are involved in spliceosome formation. The spliceosome formed in the heat-inactivated rna2 mutant extracts is functional since, upon isolation from glycerol gradients or gel filtration columns, it can splice the associated pre-mRNA in the presence of complementing extracts. Characterization of the rna2Δ spliceosome and purification of the activities required to complement it are currently underway.

N 418 CHARACTERIZATION OF A GENE PRODUCT (SEC14p) REQUIRED FOR YEAST GOLGI FUNCTION, David E. Malehorn and Vytas A. Bankaitis, Department of Microbiology, University of Illinois, Urbana, IL 61801.
We have obtained and characterized a genomic clone of SEC14, an S. cerevisiae gene whose product is required for yeast Golgi function. Gene disruption experiments indicated SEC14 to be an essential gene. Sequence analysis revealed an intron within the SEC14 gene and predicted the synthesis of a hydrophilic polypeptide of 304 residues. Immunoprecipitation experiments demonstrated SEC14p to be unglycosylated, with an apparent Mr = 35 Kd, and to fractionate predominantly as a cytosolic protein. Furthermore, we have found that the 4 previously recognized $sec14^{ts}$ alleles define a single missense mutation that converted Gly266 to Asp. Pulse-chase experiments identified SEC14p and $Sec14p^{ts}$ to be stable polypeptides at permissive temperatures (30°C), but that $Sec14p^{ts}$ became unstable when radiolabeling was performed at 37°C. Additional shift experiments demonstrated that $Sec14p^{ts}$ synthesized at 30°C, however, was not unstable upon shift to 37°C. The present data are consistent with the notion that SEC14p is a cytosolic factor that promotes yeast Golgi function.

N 419 CONTROL OF THE SACCHAROMYCES CEREVISIAE REGULATORY GENE PET494: TRANSCRIPTIONAL REPRESSION BY GLUCOSE AND TRANSLATIONAL INDUCTION BY OXYGEN, Donna L. Marykwas[1] and Thomas D. Fox,[2] Section of Biochemistry, Molecular and Cell Biology[1] and Section of Genetics and Development,[2] Cornell University, Ithaca, NY 14853. The product of the yeast nuclear gene PET494 is required to promote the translation of the mitochondrial mRNA encoding cytochrome c oxidase subunit III (coxIII). The level of cytochrome c oxidase activity is affected by several different environmental conditions which also influence coxIII expression. We have studied the regulation of PET494 to test whether the level of its expression might modulate coxIII translation in response to these conditions. A pet494::lacZ fusion was constructed and used to monitor PET494 expression. PET494 was regulated by oxygen availability: expression in a respiratory competent diploid strain grown anaerobically was one-fifth the level of expression in aerobically grown cells. However, since PET494 mRNA levels did not vary in response to oxygen deprivation, regulation by oxygen appears to occur at the translational level. This oxygen regulation was not mediated by heme, and PET494 expression was independent of the heme activator protein HAP2. The regulation of PET494 expression by carbon source was also examined. In cells grown on glucose-containing medium, PET494 was expressed at levels four- to six- fold lower than in cells grown on ethanol and glycerol. However, addition of ethanol to glucose- containing medium induced PET494 expression approximately two- fold. PET494 transcript levels varied over a four-fold range in response to different carbon sources. The effects on PET494 expression of mutations in the SNF1, SNF2, SSN6 and HXK2 genes were also determined and found to be minimal.

N 420 SORTING AND ASSEMBLY OF ALCOHOL OXIDASE FROM CANDIDA BOIDINII IN PEROXISOMES OF SACCHAROMYCES CEREVISIAE. Mark T. McCammon, Steven B. Trapp, and Joel M. Goodman, Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75235.

In order to study the function and assembly of peroxisomes, we have isolated and sequenced the alcohol oxidase gene from the methylotropic yeast, Candida boidinii. We have previously defined a pathway in vivo for import and assembly of the protein into C. boidinii peroxisomes. The protein is synthesized in the cytoplasm as a soluble monomer and becomes associated with peroxisomes over several minutes. A labile octameric intermediate is then formed which converts into the stable mature octamer. Only the N-terminal methionine is removed from the protein during its assembly. The alcohol oxidase gene, AOC, was expressed in S. cerevisiae under control of the S. cerevisiae ATP2 promoter. When alcohol oxidase was expressed in low amounts, the protein was observed to be octameric, indicating that assembly had taken place. Futhermore, approximately half of the protein was associated with peroxisomal fractions, as determined by enzyme marker assays and immunoblotting. The efficiency of import and assembly into octamer may be related to the level of expression of this protein. We hope to exploit this system further in order to understand peroxisomal assembly in both yeasts.

N 421 FACTORS INVOLVED IN THE POLYADENYLATION OF mRNA FROM THE *S. CEREVISIAE* ADR2 GENE, Claire L. Moore, Linda Hyman, and Marco Kessler, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111.

We are using a colorimetric screen to identify cis-acting sequences and trans-acting factors required for polyadenylation of ADR2 mRNAs. This assay uses a 2μ plasmid containing Gal10 promoter sequences, the first exon, the intron, and part of the second exon of the RP51 gene fused to the lac Z gene. Yeast transformed with this plasmid produce β-galactosidase and have blue colonies when grown on X-gal. Insertion of a 327 n fragment containing the ADR2 poly(A) site into the intron of the fusion gene reduces β-galactosidase expression by 95% if inserted in the orientation concordant with its use as a poly(A) site. Transcripts from this construct are polyadenylated at the ADR2 site within the intron. In the opposite orientation, the fragment has no effect on β-galactosidase production. 150 n of this region (20 n downstream and 130 n upstream of the polyadenylation site) is sufficient to signal processing. Further deletions will define the minimal sequence for polyadenylation and/or termination. Mutation of the AAUAAA 17 n upstream of the poly(A) site to AAUUCA does not increase β-galactosidase expression. RNA from this construct is being analyzed to determine if the mutation has affected polyadenylation but not termination, a situation which would still prevent expression of the reporter molecule. To identify critical nucleotides involved in processing, yeast have been transformed with plasmid mutagenized in vitro with hydroxylamine, and plasmids from blue colonies are being sequenced. We are also analyzing colonies ts for the blue phenotype after in vivo mutagenesis with EMS. Exogenously added precursor RNA containing either the ADR2 or CYC1 poly(A) site is processed in an in vitro system containing yeast extract and ATP. Mutations which affect polyadenylation in vivo are being tested in vitro.

N 422 THE YEAST PYRUVATE KINASE GENE IS SUBJECT TO DOSAGE COMPENSATION
Paul A Moore, Andrew J E Bettany & Alistair J P Brown: Department of Genetics, University of Glasgow, Church Street Glasgow, G11 5JS, UK.

In Saccharomyces cerevisiae, the wild-type pyruvate kinase (PYK1) gene is highly expressed and is believed to be important in regulating glycolytic flux. Transformation of S.cerevisiae (DBY746 or X4003-5B) with a plasmid containing the PYK1 gene on pJDB207 (a multicopy vector) generates transformants with varying levels of PYK1 mRNA. Transformants with relatively high levels of PYK1 mRNA grow slowly, but when they are grown for <100 generations on selective medium, a dramatic "wind-down" in both plasmid copy number and PYK1 mRNA level occurs concomitant with an increase in growth rate. Thus there appears to be a strong selection against over-expression of the PYK1 gene in S.cerevisiae. Analysis of a range of transformants revealed that as the copy number of the PYK1 gene is increased, there is not a parallel increase in the abundance of its mRNA. Similarly, a high level of PYK1 mRNA does not result in an analogous increase in the specific activity of pyruvate kinase. A specific regulatory mechanism for PYK1 at the translational level has been confirmed by determining the distribution of the PYK1 mRNA across sucrose density gradients of yeast polysomes relative to control mRNAs (encoding actin, ribosomal protein 1 and glyceraldehyde-3-phosphate dehydrogenase). When the intracellular level of the PYK1 mRNA is increased from wild-type levels (0.6% of total mRNA) to about 5% of total mRNA, the ribosome loading on the PYK1 mRNA decreases dramatically. Therefore, over-expression of the PYK1 gene seems to be limited at the transcriptional and translational levels.

N 423 N-TERMINAL PROCESSING OF THE ALKALINE EXTRACELLULAR PROTEASE OF YARROWIA LIPOLYTICA, David M. Ogrydziak and Sam Matoba, Institute of Marine Resources, University of California, Davis, CA 95616.
Four intracellular precursors of the alkaline extracellular protease (AEP) have been detected by immunoprecipitation. The two largest precursors have estimated molecular masses of 55- and 52-kilodaltons (kDa). The N-terminal amino sequence deduced from the DNA sequence suggests that the N-terminal region codes for a signal sequence. Preliminary radiosequencing suggests that the 55 kDa AEP precursor results from signal peptide cleavage between Ala_{15} and Ala_{16}. Beginning at Leu_{14}, there is a stretch of ten consecutive dipeptides with the sequence -X-Ala- or -X-Pro-, possible cleavage sites for dipeptidyl aminopeptidase (DPAPase) activity. N-terminal amino acid sequencing of a 52 kDa precursor secreted by a strain with an xpr6 mutation revealed the presence of at least three polypeptides. One began at the end of the dipeptide stretch, and two of the others began two and four amino acids upstream. This result and the identical mobilities and peptide maps of the 52 kDa secreted and intracellular AEP precursors strongly suggests that the intracellular 52 kDa AEP precursor results from DPAPase processing. Unlike all other reported cases where the dipeptide stretch is directly upstream of the mature polypeptide, in this case over 120 amino acids separate the dipeptide stretch and the N-terminus of mature AEP. The hypothesis that the dipeptide stretch may provide a mechanism for preventing premature activation of AEP in the secretory pathway will be tested by disrupting the dipeptide stretch by in vitro mutagenesis.

N 424 IDENTIFICATION BY cDNA CLONING OF AN ETHANOL- AND ISOFLAVONOID PHYTOALEXIN-RESPONSIVE GENE IN FUSARIUM, Christopher L. Schardl, Gil H. Choi and David A. Smith, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.
The responses of phytopathogenic Fusarium species to phytoalexin treatment were investigated by analysis of in vitro translation products of fungal mRNAs. Treatment of the bean pathogen F. solani f. sp. phaseoli (Fsp), with phaseollinisoflavan, an isoflavonoid phytoalexin of bean, resulted in the induction of a major mRNA species. A homologous mRNA was induced to high levels in F. oxysporum f. sp. cucumerinum (Foc) by treatment with ethanol. A full-length cDNA clone of this species was obtained, and northern blot analysis confirmed that the mRNA was induced by ethanol in Foc, and that its Fsp homolog was induced by phaseollinisoflavan. The sequence of the cDNA indicated an open reading frame encoding a 34,556 dalton polypeptide. Computer searches of nucleic acid and protein databases identified no homologous sequences. The characteristics of the mRNA - the translation initiation sequences and codon usage - were similar to those of highly expressed genes of Neurospora crassa. The corresponding gene in Foc (designated EIN) was single copy. The implication of the gene's induction and expression in the two Fusarium species are discussed.

N 425 DNA PLASMID AND dsRNA VIRUS-LIKE PARTICLE IN AN ISOLATE OF ALTERNARIA ALTERNATA, Hurley S. Shepherd, USDA, Southern Regional Research Center, New Orleans, LA 70179. Some isolates of Alternaria alternata cause a stunting of cotton plants by the production of a peptide toxin, tentoxin. Production of high levels of tentoxin is associated with the presence of double stranded (ds) RNA-containing virus-like particles (VLPs). A tentoxin-producing isolate of A. alternata has been found which contains both a VLP and a DNA plasmid. The plasmid was found during preparative isolation of the VLP. The dsRNA from the VLP migrates at 4.5 kbp compared to lambda DNA fragments, while the plasmid is 7 kbp. In vitro translation of the dsRNA gives a 40 kd product, which is immuno-precipitated by antibodies to tentoxin. The plasmid is linear with a blocked 5' end, based on exonuclease digestions. The plasmid seems to be localized in the cytoplasm and does not hybridize to nuclear or mitochondrial DNA or VLP dsRNA. No transcripts from the plasmid or VLP have been detected on Northern blots of total fungal RNA.

N 426 GENETIC CONTROL OF ARYLSULFATASES IN *Aspergillus nidulans*, Narendra K. Singh* and Obaid Siddiqi†, *Biology Department, Virginia Commonwealth University, Richmond, VA 23284, †Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India.

On prolonged derepression of wild-type culture of *Aspergillus nidulans*, four chromatographically separable fractions of arylsulfatase are synthesized. These fractions are designated as Fraction 0, Ia, Ib, and II. Fraction 0 is 170,000 daltons which other arylsulfatases are 80,000 daltons. Fraction II constitutes about 80% of total activity. Fraction Ia and Ib do not crossreact with anti-Fraction II.

Several arylsulfatase deficient mutants (*sulf*) were isolated. Each mutation caused reduction and/or alteration in the electrophoretic mobility of every fraction of arylsulfatase activity. Our result suggests involvement of some common element in the synthesis of Fraction I and II arylsulfatase. These mutations fall in three complementation groups and are localized on the left extended arm of chromosome V.

In arylsulfatase derepressed mutants (*sul-reg*) synthesis of arylsulfatase is not repressed by the presence of sulfur compounds in the medium. *Sul-reg* mutation fall in 6 different complementation groups, not linked to any *sulf* loci. In addition, constitutive synthesis of arylsulfatase was observed in mutants defective in enzymes of sulphur metabolic pathway. At least 12 loci have been identified which leads to constitutive synthesis of arylsulfatase. Most of these mutations appear to be involved in the biosynthesis of co-repressor(s).

N 427 TRANSLATIONAL ACTIVATION OF THE GCN4 mRNA OCCURS WHEN THE RATE OF TRANSLATIONAL INITIATION FOR MOST OTHER mRNAs IS DECREASED: IMPLICATIONS ON THE MECHANISM OF RIBOSOME REINITIATION. **Dimitris Tzamarias, Irene Roussou and George Thireos. Institute of Molecular Biology & Biotechnology, Foundation of Research and Technology, Heraclion 711 10, Crete, Greece.**

We present evidence showing that the steady state translational activation of the GCN4 mRNA in yeast, is based upon an increase in the rate of ribosome reinitiation following translation of the 5′ most proximal open reading frame located in its untranslated region. Such an increase is effected when the cellular amount of the GCN2 protein kinase is increased or when the function of the *GCD1* gene product is defective. Both conditions result in decreased steady state rates of overall protein synthesis. A dramatic but transient increase in the rate of GCN4 protein synthesis also occurs immediately after amino acids are removed from the growth medium and which is also based on increased ribosome reinitiation. This transient activation of *GCN4* mRNA translation coincides with a severe transient decrease in the rate of total cellular protein synthesis. Under all these conditions, the process that is affected is formation of 43S preinitiation complexes. These results reveal the existence of a coupling between this step in translational initiation and the mechanism of ribosome reinitiation, and suggest possible functions for the GCN2 and GCD1 gene products.

N 428 A K_2 NEUTRAL *SACCHAROMYCES CEREVISIAE* CHARACTERIZED. BRENDA D. **WINGFIELD, VALERIE *SOUTHGATE, ISAK S. *PRETORIUS AND HENDRIK J.J. *VAN VUUREN **Department of Microbiology, University of the Orange Free State Bloemfontein, South Africa, 9300. *Institute of Biotechnology and Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa, 7600.

A K_2 neutral strain *Saccharomyces cerevisiae* (USM12) was identified and characterized. The M double-stranded RNA (dsRNA) genome encodes for resistance to the K_2 killer toxin. The strain USM12 does not, however, produce any toxin. Hybridization studies show that the USM12 M genome has strong homology with the K_2 M genome. The USM12 M genome is larger than the normal K_2 M dsRNA genome. This suggests that the USM12 M genome is the result of an insertional mutation in a K_2 M genome. Protein profiles of extracellular proteins from USM12 and a cured strain are identical indicating that no proteins are secreted as the result of the presence of the M genome. This is the first characterization of a K_2 neutral strain.

N 429 CONDITIONAL MUTATIONS IN *SACCHAROMYCES CEREVISIAE* U2 snRNA . Mary Zavanelli and Manuel Ares, Jr. Department of Biology, University of California, Santa Cruz, CA 95064.

Splicing of introns from metazoan pre-messenger RNAs (pre-mRNAs) involves a large multicomponent complex, the spliceosome. The spliceosome is composed of several small nuclear ribonucleoprotein particles (snRNPs) which interact with pre-mRNA and each other. Of these, the U2 snRNP interacts with the pre-mRNA branchpoint at an early step during both yeast and metazoan splicing. We are interested in how the higher order structure of U2 snRNA is involved in the assembly and function of yeast U2 snRNP. Our approach has been to identify mutations that disrupt proprosed secondary structures and have functional consequences. Mutations in a single base pair, G53:C62, have identified a functionally important RNA-RNA interaction within U2 snRNA. The following appositions at this site give the following results: G53:G62, lethal; C53:C62, heat and cold sensitive (cs), slow growth at 30° C; A53:C62, cs on 1M KCl containing medium (YPDK); G53:U62, slow growth at 18°C on YPDK; A53:U62 and C53:G62, like wild type. Direct measurement of RNA levels for the most severe single mutations reveals that the mutant RNA is synthesized and accumulated to wild type levels at all temperatures, suggesting that loss of function is not due to low levels of RNA. To eliminate the possiblilty of an alternative pairing of these bases with the large dispensable region of U2 snRNA we have introduced the G53->C mutation into a functional U2 snRNA mini-gene lacking 958 nucleotides of internal sequences. Observation of the cs phenotype and demonstration of equivalent RNA stability will rule out alternative pairing to this large region of yeast U2 snRNA. Further studies will involve the isolation of both intragenic and extragenic suppressors of the cs lethality.