YEAST CELL BIOLOGY

James Hicks, Organizer

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Cell Cycle: Hormones and Receptors

1389 GENETIC CONTROL OF THE a CELL CYCLE BY α-FACTOR. [1,2]D. Jenness, [1]A. Burkholder and [1]L. Hartwell. [1]Dept. of Genetics, U. of Washington, Seattle, WA 98195 and [2]Dept of Molec. Genetics and Microbiology, U. of Massachusetts, Worcester, MA 01605.

The peptide, α-factor, blocks the division of a cells in the G1 phase of the cycle, it stimulates the appearance of agglutinins on the a cell surface, and it promotes morphological alterations ("schmoos") of the target a cell. Mutations in eight genes, ste2, 4, 5, 7, 8, 9, 11 and 12, result in insensitivity to α-factor (1,2), sst2 mutants are supersensitive (3). Only the ste2 mutants are specifically defective for a cell mating functions mediated by α-factor. We have found that radioactive α-factor binds reversibly to specific saturable sites on a cells. Temperature-sensitive ste2 mutants produced a binding activity at permissive temperature that was thermolabile, thus suggesting that the STE2 gene encodes the α-factor receptor. The ste2 and ste12 mutants grown under restrictive conditions produced no detectable binding activity. The ste5, 7 and 11 mutants showed appreciable numbers of α-factor binding sites and the ligand affinity was indistinguishable from that of the wild-type; these mutants are presumably defective for post-receptor functions of the response pathway. The ste4 mutants showed reduced affinity for α-factor, suggesting that the STE4 gene product leads to structural alterations of the receptor (either directly or indirectly).

Temperature-sensitive mating defects in ste2 mutants were suppressed partially by mutation sst2. This observation supports the notion that the SST2 gene product affects the α-factor receptor. Mutation sst2 also suppressed ste4 mutants when another mutation (ros1, ros2 or ros3) was present. This result corroborates the suggestion from the binding experiments that the STE4 gene product affects the α-factor receptor. Mutation ros1 was found to be allelic to mutation cdc39 which, under the appropriate conditions, blocks the cell division cycle at the α-factor arrest point (4).

Mutations at the STE2 locus lead to a variety of phenotypes. 25 ste2 mutants were selected that failed to arrest cell division in response to α-factor. Some of these mutants were also defective for agglutinin induction, whereas others remained inducible. The ste2 deletion mutant was defective for both responses: division arrest and agglutinin induction. Thus the STE2 gene differentially controls these two response pathways.

(1) MacKay, V. and R. Manney. 1974. Genetics 76: 273.
(2) Hartwell, L. 1980. J. Cell Biol. 85: 811.
(3) Chan, R. and C. Otte. 1982. Molec. and Cell Biol. 2: 11.
(4) Reed, S. 1982. Genetics 95: 561.

1390 A GENETIC AND MOLECULAR ANALYSIS OF DIVISION CONTROL IN S. CEREVISIAE, Steven I. Reed, Jill Ferguson, Jeffrey A. Hadwiger, Jeong-Yau Ho, Attila T. Lorincz, Thomas A. Peterson, Stephanie Richardson and Curt Wittenberg, Biochemistry and Molecular Biology Section, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

In Saccharomyces, cell division is controlled both in response to nutrient limitation and by mating pheromone in preparation for sexual conjugation. In each case restraint of division occurs late in the G1 interval of the cell division cycle leading Hartwell and his collaborators to propose the existence of an integrative gating event designated as "start." It has been our goal, using genetic and molecular methods, to describe "start" in molecular terms.

Mutations in four genes, CDC28, CDC36, CDC37 and CDC39, have been shown to confer conditional arrest at start. These genes have been isolated on recombinant plasmids and their products have been the major focus of our investigations. DNA sequence analysis of CDC28, CDC36 and CDC37 has allowed the prediction of a probable primary structure for each of the encoded products. Computer based comparisons detected relatedness between CDC28 and a number of protein kinases, including oncogenes of the src family, and between CDC36 and another yeast cdc gene, CDC4, as well as the avian retroviral oncogene ets. No match was found for CDC37. Antibodies prepared against the CDC28 and CDC36 products have been used as both biochemical and cytological probes. Immunoprecipitates from yeast cell lysates prepared with anti-CDC28 product antibody were found to contain a 34 kD phosphoprotein, shown to be the CDC28 product. These immunoprecipitates, in addition, were found to possess a protein kinase activity. Phosphate was transferred from exogenous ATP to a 40 kD polypeptide which had co-precipitated with the CDC28 product. The reaction was thermolabile when temperature sensitive CDC28 mutants were used. Preliminary evidence suggests that the 40 kD polypeptide is the in vivo substrate of a CDC28-encoded protein kinase. Fluorescent antibody double labelling experiments indicate that much, if not all, of the CDC28 product is associated with actin fibers of the yeast cytoskeleton. Furthermore, genetic studies suggest that the CDC28 product functions in controlling actin structure. We propose that the start event may constitute a cytoskeletal transition mediated, at least in part, by protein phosphorylation. The implications of this model with respect to cell cycle regulation and the possible roles of other start gene products will be discussed.

Cell Cycle, Hormones and Receptors

1391 IDENTIFICATION OF A GLYCOPROTEIN INVOLVED IN THE G_1 TO S TRANSITION IN YEAST.

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A labile protein p100 (MW=100 Kd, pI ~4.8-5) is specifically made during release from cell cycle arrest of the mutant cdc25 of S.cerevisiae which is blocked in the start area at nonpermissive temperature. Another labile protein p100*, contiguous to p100 on 2D-gels, behaves as a cell cycle modulated protein preferentially made during late G_1 till mid S phase. Pulse-chase labeling experiments and peptide mapping analysis indicate that p100 and p100* are processed to a protein p115 (115 Kd, pI~4.8-5.2). By labeling experiments with ^{3}H-glucosamine and by 2D analysis of total protein, p115 results to be the major yeast glycoprotein. The synthesis of p100, p100* and p115 is inhibited by tunicamycin. P115 is localized in the plasma membrane and its biochemical function is now under investigation.

1392 GENETIC STUDIES ON THE MECHANISM OF VACUOLE ASSEMBLY IN YEAST. Vytas A. Bankaitis and Scott D. Emr, Division of Biology, California Institute of Technology, Pasadena, CA 91125

We are studying the mechanism by which proteins are delivered to the vacuole in the yeast Saccharomyces cerevisiae. To this end, we have constructed fusions of PRC1, the gene encoding the vacuolar enzyme carboxypeptidase Y (CPY), to SUC2, the gene encoding a normally secreted yeast enzyme invertase. These hybrid genes program the synthesis in yeast of chimeric proteins that consist of amino-termini derived from CPY and a carboxy-terminus derived from invertase. Such chimeric proteins exhibit invertase activity. Our data indicate that relatively short portions of the amino-terminal CPY primary sequence are sufficient to result in the delivery of CPY-invertase fusion proteins to the vacuole. Fusion strains are consequently unable to utilize sucrose as a sole carbon source. We have exploited this property to obtain mutants that are defective in the targeting of CPY-invertase to the vacuole and instead secrete a large percentage of the hybrid protein into the yeast periplasm. The corresponding mutations are all trans-acting, recessive, and define at least eight complementation groups. Our experiments indicate that such mutants are similarly defective in the localization of wild-type CPY to the yeast vacuole and that, in these mutant strains, CPY accumulates in its core glycosylated form. We believe that the gene fusion approach and an analysis of such vacuolar protein targeting (VPT) mutants will contribute to an understanding of how proteins are sorted in yeast.

1393 ISOLATION OF THE GENE FOR HMG-CoA REDUCTASE: THE RATE-LIMITING ENZYME OF STEROL BIOSYNTHESIS. Michael Basson, Mary H. Krall, and Jasper Rine, University of California, Berkeley CA 94720

3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMG-CoA reductase) is the rate-limiting enzyme for the biosynthesis of sterols and all other isoprenoid-derived compounds, such as dolichol, ubiquinone, and isopentenylated adenosine. We isolated genes that increase HMG-CoA reductase activity by selecting from a population of cells transformed with a yeast genomic library on multicopy plasmids those that were resistant to a competitive inhibitor of HMG-CoA reductase. The plasmids isolated from resistant transformants contained overlapping pieces of the yeast genome. Cells containing these plasmids contain 8-14 times more HMG-CoA reductase activity than do isogenic cells without the plasmid.

DNA-RNA hybridization blots using pieces of the insert DNA as probes indicate the presence of three transcripts. By deletion mapping we established that a 3.3 kb RNA is responsible for overproduction of enzyme activity. To determine whether this gene is the structural gene for HMG-CoA reductase, we performed low stringency DNA-DNA hybridization using a cDNA for hamster HMG-CoA reductase as a probe. This cDNA cross-hybridized with a 574 bp fragment of yeast DNA from the region encoding the 3.3 kb RNA. Translation of the open reading frames in the yeast and hamster genes would yield proteins in which long stretches of amino acid residues are conserved in the region of the active site of the enzyme. This sequence homology strongly suggests that the yeast gene is the structural gene for HMG-CoA reductase. Studies of the regulation of this gene are in progress.

1394 a-FACTOR STRUCTURAL GENES, Charles Brenner, Richard Najarian, Paul Laybourn, James Merryweather and Anthony Brake, Chiron Corp., 4560 Horton St., Emeryville CA 94608.
Two segments of yeast genomic DNA were isolated from a plasmid library by hybridization to synthetic oligodeoxynucleotide pools complementary to DNA sequences encoding the reported amino acid sequences of mating pheromones secreted by S. cerevesiae a-cells. These two fragments appear to contain functional genes since they hybridize to mRNA a-cell specifically and, on multicopy plasmids, increase the level of a-factor secreted by MATa transformants. Transcriptional control is thought to be located within DNA sequences upstream of the a-factor coding regions where the two fragments have strong homology with other a-specific and haploid-specific genes. The fragments encode 36 and 38 residue peptides which contain the reported undecamer sequences with a change in the order of the 3 C-terminal residues, and with the addition of 4 amino acids C-terminal to the reported terminus, suggesting that these residues are removed either in normal peptide processing or as an artifact of a-factor purification. The coding sequences diverge from one another little but are unlike the structure of most precursors to secreted proteins in that the C-terminal (mature pheromone-containing) portion is hydrophobic while the N-terminal portion is not. Genetics and the deduced a-factor precursors' sequences suggest that their processing and secretion pathway is distinct from that of α-factor precursors. The small size and lack of signal sequences in these putative a-factor precursors suggest that they may be post-translationally inserted into the endoplasmic reticulum.

1395 GENETIC ANALYSIS OF THE α-FACTOR RECEPTOR, Anne C. Burkholder and Leland H. Hartwell, Dept. of Genetics SK-50, University of Washington, Seattle, WA 98195.

MATa cells specifically bind α-factor and respond to it by arresting cell division, inducing cell surface agglutinins and forming an altered morphology described as projection formation. Temperature-sensetive mutations in the a-specific STE2 gene result in thermolabile α-factor binding activity. A STE2 null mutant (ste2-10::LEU2, constructed by substitution of the genomic STE2 gene with LEU2) has no α-factor binding activity, is completely sterile, and shows no response to α-factor in assays for cell division arrest, agglutinin production, or projection formation. These mutant phenotypes suggest that the function of the STE2 gene is to encode a structural component of the α-factor receptor.

The analysis of the α-factor dose response and binding activity of STE2 mutants that retain partial activity indicates that the receptor is functionally complex. One mutant, ste2-8, has very little α-factor binding activity and does not division arrest or form projections even at high α-factor concentrations, yet has a nearly wild-type dose response for the induction of agglutinin. The ste2-9 mutant does not bind α-factor, division arrest or form projections, but has a high constitutive level of agglutinin. The STE2 gene product may have different functional domains responsible for binding α- factor and signaling the responses which can mutate independently. We are trying to map these domains by analyzing the positions and phenotypes of additional mutants in the STE2 gene.

1396 CDC17 FUNCTIONS IN TELOMERE METABOLISM, Michael J. Carson and Leland H. Hartwell, University of Washington, Seattle, WA 98195

The CDC17 gene performs an essential function during the cell cycle. Strains with a temperature sensitive allele of cdc17 show elevated levels of mitotic recombination at sublethal temperatures. We have mapped these recombination events and find that they occur preferentially in the intervals of the chromosome closest to the telomere. This result suggested a defect in DNA metabolism near the end of the chromosome. We find that telomeres are longer in cdc17 strains than in CDC17 strains at the permissive temperature and that they become even longer by addition of sequence near or in the C[1-3]A telomeric repeated DNA when cells are propagated at sub-lethal temperatures. Mutations in other genes that function at a similar stage of the cell cycle have no effect on telomere length. The altered telomere length phenotype of the cdc17-1 mutation is recessive and coreverts and cosegregates with the temperature-sensitive lethal phenotype; we conclude that both phenotypes result from a deficiency in the amount of functional CDC17 gene product. Although the mitotic recombination events induced in cdc17-1 strains requires RAD52 function, telomere growth occurs in a rad52 mutant. Thus telomere growth is not a consequence of rad52 dependent mitotic recombination. We conclude that the CDC17 gene product probably plays some essential role in telomere metabolism and is necessary to prevent telomeres from growing longer during cell division.

1397 TRANSCRIPTION OF CDC GENES IN S. POMBE, Peter A. Fantes, David Hughes and Colin Gordon, Edinburgh University, Edinburgh EH9 3JT, U.K.

We are investigating the transcription pattern of cdc genes during the normal cell cycle and following cell cycle arrest. The transcription of three cloned genes is currently under study: cdc 27, a gene required for mitosis; cdc 22, required for DNA replication; and an extragenic suppressor of cdc 22 mutations. Our results so far show that cdc 22 expression is not dependent on continued cell cycle progress.

Further observations on the transcription of these and other genes will be presented.

1398 CELL TYPE-SPECIFIC CONTROL OF MFα1 EXPRESSION IN YEAST, Monica C. Flessel and Jeremy Thorner, Department of Microbiology and Immunology, University of California, Berkeley, California 94720.

MFα1, the major structural gene for α-factor mating pheromone in Saccharomyces cerevisiae, is expressed only in MATα cells. The current model for such cell type-specific control of gene expression suggests that MFα1 and other α cell-specific genes require positive regulation, either directly or indirectly, by the protein product of the MATα1 locus. Sequence comparisons between the 5'-flanking regions of two α cell-specific genes, MFα1 and STE3, have revealed consensus sequences that may represent potential regulatory sites.

We have been investigating the cell type-specific control of MFα1 expression utilizing an MFα1-SUC2 gene fusion which places invertase expression under the control of the MFα1 promoter. We have compared levels of expression of invertase activity in all three yeast cell types (MATa and MATα haploids, and MATa/MATα diploids) when the fusion gene is carried on a multi-copy plasmid, on a centromeric plasmid, or is integrated into the genome in replacement of the wild-type MFα1 locus. We have constructed promoter mutations in vitro and assayed for corresponding changes in the level and cell type-specificity of invertase activity. Deletions generated with Bal 31 nuclease indicate that a 50 bp region containing two direct repeats of a short palindromic sequence (CTAATTAG) is important for high level and α cell-specific gene expression. We are currently utilizing linker-insertion mutagenesis to generate small deletions and insertions, and chemical mutagenesis to generate point mutations, to further define the important features of these regulatory regions.

1399 MOLECULAR ANALYSIS OF THE STE5 GENE PRODUCT, A HAPLOID-SPECIFIC FUNCTION REQUIRED FOR PHEROMONE-INDUCED CELL CYCLE ARREST. Richard Freedman, Janet Schultz and Jeremy Thorner, University of California, Berkeley, California 94720.

We previously isolated the STE5 gene from a genomic library and localized the gene within a 4 kb region of the original insert by deletion mapping and sub-cloning. Electrophoretic analysis of polyA$^+$ RNA using a STE5-specific DNA probe demonstrated that the gene specifies an mRNA of about 2.9 kb. This transcript is found in both MATa and MATα haploids, but not in MATa/MATα diploids; the transcript is found, however, in matα1/MATα and MATa/matα2 mutant diploids, suggesting that STE5 is under the negative transcriptional control of the MAT regulatory proteins. We have determined the complete nucleotide sequence of over 3600 bps of the STE5-containing fragment. This region contains an open reading frame of 2751 bps, with no apparent introns, that could potentially encode a hydrophilic polypeptide of 103,000 MW. Preceding the ORF, at about -200 bps upstream from the ATG, are repeats of a sequence, the so-called "a/α box", that has been found in multiple copies in the 5'-flanking region of other yeast genes whose transcription is repressed in diploids (including HO, MATα1 and certain Ty elements) and which appears from the work of others to be sufficient to repress transcription of downstream genes in MATa/MATα cells. Even when STE5 is carried on a multi-copy plasmid, presence of the a/α box sequences is sufficient to completely repress expression in diploids. To study its regulation and to prepare antibodies for determining the intracellular location and function of its product, the STE5 gene has been fused to both the yeast SUC2 gene and the E. coli lacZ gene.

1400 COMPARATIVE STUDY OF THE YEAST RAS1 AND RAS2 PROTEINS, Asao Fujiyama, Madan Rao, Nasrollah Samiy, and Fuyuhiko Tamanoi, Cold Spring Harbor Laboratory, NY 11724

The RAS1 and RAS2 genes of Saccharomyces cerevisiae are the close homologs to the mammalian ras genes (Powers et al. 1984, Cell, 36, 607). Genetic analyses have shown that neither RAS1 nor RAS2 are essential genes; however, double mutation in these genes inhibits continuous growth of the mutant (Kataoka et al. 1984, Cell, 37, 437. Tatchell et al. 1984, Nature, 309, 523). Recent analyses of the mutants have suggested the participation of the RAS protein in the regulatory mechanism(s) of adenylate cyclase activity in yeast (Toda et al. 1984, Cell, in press). We have been carrying out biochemical and structural studies on the yeast RAS proteins. The RAS2 protein overproduced in yeast cells under the control of GAL10 promotor exhibited GDP and GTP binding, which is characteristic of other ras proteins (Tamanoi et al. 1984, P.N.A.S., in press). Although the amino acid sequences of RAS1 and RAS2 proteins are very similar in the first 180 residues, they diverge in the C-half portions, indicating that each might function differently in yeast. To investigate this possibility, we have started biochemical analysis of the RAS1 protein. First, we constructed overproducing systems. Cloned RAS1 DNA fragments were inserted downstream of the lac promotor for the expression in E.coli or of the GAL10 promotor for S.cerevisiae. The RAS1 protein purified from E.coli to near homogeneity has molecular weight of 32-33Kd (SDS-PAGE). The RAS1 protein binds both GDP and GTP at nearly the same level as RAS2 protein. Furthermore, the RAS proteins have GTPase activities.

1401 FUNCTION OF THE ras-RELATED YP2 GENE IN SACCHAROMYCES CEREVISIAE, Dieter Gallwitz, Cornelia Donath, Hans-Joachim Perski, Andreas Rauh, Hans-Dieter Schmitt and Peter Wagner, Institut für Physiologische Chemie, Universität Marburg, D-3550 Marburg, FRG

Three ras-related genes have been found in Saccharomyces cerevisiae. One of them, the YP2 gene (1), is a single copy gene and serves a vital function. Gene disruption shows that the other two ras-related genes, RAS1 and RAS2, cannot complement the YP2 gene defect. Mutations introduced into the protein coding region have enabled us to identify specific amino acid residues critical for the normal functioning of the YP2 protein. Data will also be presented on the regulation of the YP2 gene expression.

(1) Gallwitz,D., C.Donath & C.Sanders (1983) Nature 306, 704-707.

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1402 GENETIC ANALYSIS OF MICROTUBULES, Tim C. Huffaker, James H. Thomas and David Botstein, M.I.T., Cambridge, MA 02139

S. cerevisiae contains a single essential gene (TUB2) encoding β-tubulin. Mutations in TUB2 have been isolated by selecting cells resistant to the anti-mitotic drug benomyl. Included among these were 9 conditional-lethal mutants, 3 temperature-sensitive (Ts) and 6 cold-sensitive (Cs) for growth. We have also used the more general technique of in vitro mutagenesis followed by gene replacement to isolate 4 Ts and 5 Cs mutations in TUB2 which do not confer resistance to benomyl. Strains carrying these TUB2 mutations showed a cell-cycle specific arrest at their restrictive temperatures as large-budded cells blocked in mitosis. Incubation of strains carrying the Cs allele tub2-104 at the restrictive temperature resulted in depolymerization of most of the cell's microtubules as visualized by indirect immunofluorescence. DAPI staining of the nuclear DNA showed that 90% of the large-bud arrested cells contain a single unelongated nucleus. These observations are consistent with a role for microtubules in chromosome segregation in yeast. The tub2-104 mutation also confers a cold-sensitive defect in nuclear fusion during conjugation and a recessive defect in sporulation. We expect that many suppressors of TUB2 mutations will compensate for the tubulin defect by altering an interacting protein. This approach should allow us to identify microtubule associated proteins. Twenty seven mutations in 16 complementation groups have been identified that suppress the Cs phenotype of tub2-104 and themselves confer a Ts lethal phenotype. At least one of these groups is likely to directly affect microtubule function, since its Ts phenotype is very similar to the Ts alleles of TUB2. We also intend to present data on the other TUB2 alleles and their suppressor groups.

1403 CYTOSKELETON OF SCHIZOSACCHAROMYCES POMBE, Jeremy S. Hyams, John Marks and Iain Hagan, Department of Botany & Microbiology, University College London, Gower Street, London WC1E 6BT. U.K.

Changes in the organization of tubulin and actin through the cell cycle of the fission yeast were investigated by fluorescence microscopy using anti-tubulin antibody (Kilmartin) and phalloidin (Wiehland) as specific probes. Microtubules were visualized as two distinct arrays; during interphase, cytoplasmic microtubules extended parallel to the long axis of the cell whilst at division they formed a brightly staining spindle. Actin staining was mainly in the form of dots, the distribution of which coincided precisely with the known pattern of cell wall deposition in this organism (Mitchison and Nurse, in press). In short cells (<9.5 μm), dots were seen only at the old end (the end which existed prior to septation). Above this length double end staining was observed until the cell entered mitosis when a ring of actin anticipated the formation of the septum. Arrays of actin dots were also seen to be spatially associated with the multiple septa produced when the cell division cycle mutant cdc 13 was grown at the restrictive temperature.

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1404 IDENTIFICATION OF AN ELEMENT UPSTREAM FROM THE α-SPECIFIC STE3 GENE OF YEAST THAT IS SUFFICIENT FOR REGULATION BY MATING TYPE. Eric E. Jarvis*, David C. Hagen*, George F. Sprague, Jr.*, Debra Chaleff+, *University of Oregon, Eugene, OR 97403, +E.I. DuPont Co., Wilmington, DE 19898.

The expression of the STE3 gene of S. cerevisiae is controlled in at least three ways: 1) STE3 RNA is present in α cells, not in a or a/α cells; α-specific activation requires the α1 protein encoded by MATα. 2) The levels of STE3 RNA increase fourfold when α cells are exposed to a-factor. 3) The products of STE7 and STE11 are required for efficient expression. Deletions approaching the STE3 gene from upstream indicate that sequences as far as 450 base pairs 5' to the ATG of STE3 are required for proper regulation. To define the 3' limits of these sequences, we have used a plasmid containing a CYC1-lacZ gene fusion (provided by L. Guarente) into which varying lengths of STE3 upstream sequences are inserted in place of the upstream activation sites (UAS's) of the CYC1 gene. Transcriptional and translational start signals are provided by CYC1 sequences. So far it has been demonstrated that a piece of DNA ~170 base pairs in length is sufficient to confer α-specificity and a-factor inducibility onto the CYC1-lacZ gene. Preliminary results using ste7 and ste11 deletion mutants indicate that the products of STE7 and STE11 are required for the expression mediated by these upstream sequences. Computer analysis of STE3 upstream sequences indicates that the 170 b.p. fragment contains at least one sequence that is also found in another α-specific gene, a structural gene encoding α-factor (MFα1). Four similar copies of this 13 b.p. STE3 sequence are located upstream from the MFα1 gene at a comparable distance.

1405 A G-0 MUTANT OF YEAST, Gerald C. Johnston, Michael A. Drebot and Richard A. Singer, Dalhousie University, Halifax. NS B3H 4H7, Canada

A non-dividing cell may enter what has been termed the G-0 state, in which it is physiologically distinct from a proliferating cell. For yeast this G-0 state can be equated with stationary phase. We have isolated a mutant strain of S. cerevisiae conditionally defective for outgrowth from stationary phase. When mutant cells first grown to stationary phase at the permissive temperature of 30° were shifted to fresh medium at the restrictive temperature of 14°, cell division did not occur for at least 8 generation times. Those cells which did eventually proliferate no longer showed the cold-sensitive outgrowth defect, suggesting that they were pseudorevertant cells which overgrew the non-dividing mutant cell population. The defect is specific for outgrowth from stationary phase, since exponentially growing cells shifted to 14° continued to divide exponentially for at least 6 generations. Genetic segregation patterns suggest that the defect is multigenic. This mutant phenotype is evidence that the G-0 state may be a genetically distinct cell cycle phase not experienced by proliferating cells.

1406 GENES SELECTIVELY EXPRESSED DURING PROLIFERATION OF DICTYOSTELIUM AMOEBAE. W. Kopachik, L. G. Bergen and S. L. Barclay, University of Wisconsin, Madison, WI 53706.

From a cDNA library of log phase Dictyostelium discoideum we identified transcripts which are abundant in proliferating but are at low or undetectable levels in non-proliferating developing cells. Plasmids from nine representative clones had unique restriction fragment patterns and hybridized to different-sized mRNAs. These sequences displayed a range of species homologies when hybridized to D. purpureum or D. mucoroides RNA and four showed strong homologies to transcripts from Polysphondylium violaceum. Accumulated levels of these transcripts decreased to undetectable levels during development. Mature spores contained no detectable mRNA homologous to these cDNAs, although actin mRNA was shown to be present. Synchronously germinating spores contained no homologous mRNA at one hour post induction, but abundant levels of mRNA homologous to all cDNAs were found at 3 hr post induction. The high level of expression of these transcripts prior to the first cell division after germination, and their shut off during differentiation, suggests that they have an important role in proliferation of amoebae.

1407 BIOSYNTHESIS AND METABOLISM OF ISOPENTENYLADENOSINE IN S. CEREVISIAE, Howard M. Laten and Carol A. Van Kast, Loyola University of Chicago, Chicago, IL 60626

Isopentenyladenosine is a member of a class of potent plant growth hormones called cytokinins. It (or a related derivative) is also a constituent of tRNA species in plants, animals, and microorganisms. The presence of free isopentenyladenosine in plants has been attributed to tRNA turnover, de novo synthesis, or both, and the existence of free isopentenyladenosine in slime mold and yeast has only recently been demonstrated. Its function in yeast is not known. We have shown that free isopentenyladenosine is synthesized de novo in yeast (Laten et al., PNAS, in press). We have further characterized this synthesis and present evidence from in vivo labelling studies that AMP is one of the substrates in the reaction. Degradation of isopentenyladenosine has been shown to be catalyzed by adenosine (or adenine) aminohydrolase in fission yeast and mammals, and by cytokinin oxidase in plants. Our results from genetic and in vivo labelling studies involving isopentenyladenine utilization by adenine auxotrophs suggest that cytokinin oxidase catalyzes the conversion of isopentenyladenine to adenine in S. cerevisiae.

1408 INTERACTION OF YEAST SEXUAL AGGLUTININS. Peter N. Lipke, Kevin Terrance, Neil Wagner, and Yu-Sheng Wu, Hunter College of CUNY, New York, NY 10021

Sexual agglutinins of S. cerevisiae have been partially purified from cellular extracts. The a-agglutinin, purified at least 160-fold, is 95% carbohydrate, is heat stable, and is inactivated by $NaIO_4$ or pronase. It is rich in Ser, Gly, Glx, and Ala. α-Agglutinin, purified at least 500-fold, contains several glycopeptides associated with activity. 30% of the weight is carbohydrate. The interaction of the agglutinins has been studied by cellular bioassays and by binding of ^{125}I-labelled α-agglutinin. Both assays suggest that a-agglutinin is the α-agglutinin receptor and vice versa. The agglutinins appear to interact in either of two states, one with a K_a of 10^9 L/MOL and the other considerably weaker. The two states form with similar kinetics, and are similarly affected by perturbations of pH, temperature, and ionic strength. The ratio of binding in the two states is not altered by pretreatment of a-cells with α-factor. The structural basis for the binding heterogeneity is not known. Binding assays demonstrate that there are about 10^4 α-agglutinin receptors per a-cell, the number being increased 2 to 4 fold following challenge with α-factor. (Supported by grants from NSF and the CUNY PSC-BHE program)

1409 IDENTIFICATION OF GENES REGULATED BY MAT AND BY MATING PHEROMONES. Gretchen McCaffrey and George F. Sprague, Jr., Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, OR 97403

The yeast Saccharomyces cerevisiae displays three cell types: α, a, and a/α. Positive and negative regulators encoded by the mating type locus alleles (MATa and MATα) affect the expression of unlinked genes, the products of which are responsible for the characteristic traits of the different cell types. The expression of some of these genes is also affected by the yeast mating pheromones, α-factor and a-factor. To identify common features among genes regulated either by the MAT alleles or by the pheromones, we have isolated control sequences from genes which are expressed only in α cells (α specific), or in α and a cells but not in a/α diploid cells (a/α inhibited). This isolation has been accomplished by screening a lacZ fusion library (provided by S. Fields and K. Wilson). From 368,000 transformants we have identified 81 plasmids which produce β-galactosidase in α cells but not in a/α diploid cells. At least 46 of these plasmids produce the same amount of β-galactosidase in a cells as they do in α cells and are therefore a/α inhibited. We have not yet identified any new α-specific genes. LacZ expression in some of these plasmids is affected by a-factor treatment.

In the two cases examined so far, RNA production from the chromosomal gene is regulated in the same manner as the plasmid-borne lacZ is regulated. These two genes are not known a/α inhibited genes; their restriction patterns or transcript sizes differ from those of HO, Ty1, Ty912, STE5, MATα1 and MATα2.

1410 CYCLOHEXIMIDE RESISTANT CELL DIVISION CYCLE MUTATIONS. John McCusker and James E. Haber, Brandeis University, Waltham, MA 02254

Spontaneous cycloheximide resistant mutations were selected at 1μg/ml cycloheximide and were screened for ts lethality. 6-7% were found to be ts lethal. The crl (cycloheximide resistant lethal) mutations are ts mutations in 22 essential genes which appear to be functionally related. All of the mutations arrest late in the cell cycle at 37°C, fail to arrest in G1 in stationary phase or in response to amino acid starvation, are hypersensitive to trifluoroleucine, β-thienylalanine and thialysine; are hyposensitive to canavanine. Anaerobic conditions phenotypically suppress the cycloheximide resistance of all but one of the crl mutations at 25°C and at least partially phenotypically suppress the ts lethality of most of the crl mutations. Other phenotypes associated with some of the crl mutations include: cross resistance to structurally and functionally unrelated antibiotics, sporulation defects (spo⁻ or 2 spored asci), hyperaccumulation of glycogen. Data will be presented concerning mapping, the nature of the sporulation defects and nuclear morphology.

1411 INTRACELLULAR cAMP AND cGMP CONCENTRATIONS IN SPORULATING YEAST SACCHAROMYCES CEREVISIAE. Zofia Olempska-Beer, National Institutes of Health, Bethesda, MD 20205. It has been suggested that a decrease of cAMP may be necessary for the initiation of meiosis and sporulation in Saccharomyces cerevisiae. I have measured the intracellular concentrations of cAMP and cGMP during sporulation of the wild type strain Y55 and a guanine auxotroph. The sporulation was initiated by transferring cells growing exponentially in a gluconeogenic medium to a medium containing either a growth limiting concentration of pyruvate or one lacking either nitrogen or sulfur (for Y55) or guanine (for guanine auxotroph). No consistent pattern of changes in cAMP concentration was observed. However, cGMP (and GTP) always decreased after transfer to the sporulation medium, sometimes after a brief initial increase. These results suggest that a decrease in the concentration of cAMP is not required for meiosis and sporulation, whereas a decrease of GTP or cGMP may be necessary.

1412 TWO FUNCTIONAL ALPHA-TUBULIN GENES IN SACCHAROMYCES CEREVISIAE, Peter J. Schatz and David Botstein, Massachusetts Institute of Technology, Cambridge, MA 02139

We have isolated two different genomic DNA sequences from the yeast Saccharomyces cerevisiae that are highly homologous to the two alpha-tubulin genes of the fission yeast Schizosaccharomyces pombe (generously provided by T. Toda and M. Yanagida). DNA sequence studies, as yet incomplete, reveal open reading frames that encode amino acid sequences strongly homologous to alpha-tubulins from other species. The two genes have been called TUB1 and TUB3 (TUB2 encodes beta-tubulin). Both alpha-tubulin genes appear to have intervening sequences beginning in codon 9.

Gene disruption studies show that TUB3 is not essential for vegetative growth, mating or sporulation, although some deleterious effects are observed. These effects, as well as an increase in sensitivity to the antimicrotubule drug benomyl show that the TUB3 gene is expressed. Some spores containing an apparently disrupted TUB1 gene have also been recovered, although the frequency and the growth and sporulation phenotypes of diploids heterozygous for such a disrupted gene suggest that TUB1 might be more important in both growth and sporulation. A fuller characterization of the structure, function and expression of the yeast tubulin genes is in progress.

1413 GENETIC STUDY OF THE YEAST YP2 GENE: A PUTATIVE ras PROTO-ONCOGENE HOMOLOGUE, Nava Segev and David Botstein, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The yeast gene, YP2, encodes a protein that exhibits significant homology to the mammalian ras proteins. This gene is located between the actin (ACT1) and beta tubulin (TUB2) genes, on chromosome VI, and is expressed in growing cells. YP2 is not a member of the complementary set of RAS1 and RAS2 genes, the two close yeast homologs of the ras oncogenes. Using gene disruption techniques we have shown that the intact YP2 gene is required for spore viability. Using recessive conditional lethal mutations in the actin and tubulin genes, we have also shown that these genes, which flank YP2, are functional in diploid cells that contain a YP2 disrupted genes in cis configuration. Thus, we can conclude that the YP2 gene product is essential for the vegetative growth of yeast. Unlike RAS1 and RAS2 genes, the YP2 gene does not seem to work through the adenylate cyclase regulatory system. We have shown that by disrupting the YP2 gene in a bcy1 cell. Cells with bcy1 mutations have a continuous activation of protein kinase which is normally activated by cyclic AMP. This mutation can compensate for the RAS1 and RAS2 lack of activity, but it cannot bypass the YP2 gene product. In order to better understand the biological role of the YP2 gene product in cell growth and to investigate its possible importance in the cell cycle, conditional-lethal mutants are being constructed by in vitro mutagenesis.

1414 GENETIC INSTABILITY OF THE pep4 MUTATION. Jeffrey R. Shuster, Anne Randolph and Carlos George-Nascimento, Chiron Corporation, Emeryville, CA 94608

Use of pep4 mutant strains of Saccharomyces cerevisiae have proven useful in the reduction of generalized protease activities during the isolation of proteins from yeast. We have found that the reduction in carboxypeptidase-Y (cpY) activity due to the pep4-3 mutation becomes unstable during prolonged subculturing on YEPD complex medium. Three pep4-3 strains were tested for cpY activity after approximately six months of subculturing. The strains contained 38%, 14%, and 89% of the cells in the populations as cpY^+ respectively. Further analysis of one of the cpY^+ clones indicated that the strain had picked up a suppressor mutation which also restored wild type levels of protease-B to the cells. Therefore, it is recommended that users of pep4 strains monitor the strains periodically for reversion. Data will be presented demonstrating the effect on protein production in a pep4-3 and a reverted strain.

1415 CELL CYCLE REGULATION OF GROWTH IN YEASTS, Richard A. Singer and Gerald C. Johnston Dalhousie University, Halifax, NS B3H 4H7, Canada.

Cellular activities during proliferation have been grouped into two classes: the DNA-division sequence comprising the programmed sequence of periodic events for replication and segregation of nuclear DNA, and "growth" activities involved in the production of new cell mass. Growth is normally rate-limiting for cell proliferation. Growth and the DNA-division sequence are necessarily coordinated, in part by growth requirements for certain DNA-division sequence steps. Another form of coordination has been recently recognized to occur in the fission yeast S. pombe; in those cells growth seems to be regulated by the DNA-division sequence. This latter, novel coordination has been manifested by growing S. pombe cells under limiting conditions which make performance of the DNA-division sequence, not growth, the rate-limiting determinant for cell proliferation. Relief of such limiting conditions for S. pombe cells allowed accelerated growth, in conjunction with accelerated cell division. Cells of the budding yeast S. cerevisiae, however, did not show this coordination; relief of such limiting conditions allowed accelerated cell division, but without accelerated growth. Moreover, under such limiting conditions populations of smaller S. cerevisiae cells produced by slowing growth with cycloheximide gave rise to larger cells when cycloheximide was removed. Those observations suggest that in S. cerevisiae, unlike S. pombe, growth is not regulated by the DNA-division sequence.

1416 ROLES OF CYCLIC AMP-DEPENDENT PHOSPHORYLATION AND DEPHOSPHORYLATION OF PROTEINS IN THE YEAST CELL CYCLE, Isao Uno, Kunihiro Matsumoto*, and Tatsuo Ishikawa, Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, Japan;*Tottori University, Tottori, Japan

The regulatory roles of cAMP in Saccharomyces cerevisiae have been studied by using mutants defective in adenylate cyclase or cAMP-dependent protein kinase. We demonstrated that cAMP-requiring mutants arrested at the G1 phase of the cell cycle in the absence of cAMP, and that phosphorylation of cellular proteins by cAMP-dependent protein kinase has essential functions in passing through G1 to S phase of the cell cycle. Phosphoproteins are dephosphorylated with phosphoprotein phosphatases. The ppd1 mutant was isolated as a suppressor of the cyr2 mutant which has the altered catalytic subunit of cAMP-dependent protein kinase. At least three kinds of phosphoprotein phosphatase (I, II and III) were found in crude extracts of the wild type strain. The ppd1 mutant was deficient in type I phosphoprotein phosphatase activity. The ppd1 mutant characteristics suggest that the decreased phosphorylation of cellular proteins in the cyr2 mutant may be overcome by the decreased dephosphorylation in the absence of type I phosphoprotein phosphatase activity. The ppd1 and bcy1 mutants which produce high level of cAMP-independent protein kinase suppress G1 arrest by the nutritional limitation orby the adenylate cyclase mutation (cyr1). The results suggested that phosphorylation of cellular proteins is indispensable for cell cycle, but dephosphorylation is not.

1417 SIGMA ELEMENTS MAY FUNCTION AS HORMONE-RESPONSIVE PROMOTERS, Scott W. Van Arsdell, Gary L. Stetler and Jeremy Thorner, University of California, Berkeley, CA 94720.

We have previously isolated several genomic clones containing sequences whose expression in a cells is altered after exposure to α-factor. Detailed analysis of clone ScG7 indicates that it encodes a 650-base polyA$^+$ RNA species which is at least 100 times more abundant in a cells which have been treated with α-factor compared to untreated a cells. The 650 base RNA is transcribed from a region consisting of a cluster of short repetitive elements. S1 mapping indicates that transcription initiates within a full-length sigma element and terminates within a full-length delta element located downstream. When a single-stranded probe containing only sequences from the ScG7 sigma element is used in Northern blot analysis, three major polyA$^+$ RNA species of approximately 450, 650, and 4900 bases are detected. Each of these RNAs is dramatically induced by α-factor. The three sigma-related transcripts show similar levels of induction in a cells treated with α-factor and in α cells treated with a-factor; none of the three RNAs are present at significant levels in diploids. We have recently constructed a recombinant plasmid in which the ScG7 sigma element has been inserted just upstream of the coding sequence and terminator of a promoter-less SUC2 gene. In a cells transformed with the sigma-SUC2 fusion plasmid, a new prominent 1.8 kb polyA$^+$ transcript appears in response to α-factor which hybridizes to both sigma and SUC2 probes. In vitro mutagenesis techniques are being used to map precisely the sequences that are required for this hormonal induction of transcription. These data suggest that at least a sub-set of the 25-30 sigma elements in the yeast genome function as hormone-responsive promoters which turn on the expression of downstream genes during the mating process.

1418 IDENTIFICATION OF A MYOSIN-LIKE PROTEIN IN SACCHAROMYCES CEREVISIAE. F.Z. Watts, E. Orr, Dept. of Genetics, University of Leicester, University Road, Leicester, U.K.

We have now identified a myosin-like protein in Saccharomyces cerevisiae. This protein with a molecular weight of approximately 200K has been characterised biochemically and immunologically. Evidence suggesting that the sequence of this protein is relatively conserved through evolution will be presented. Antibodies against this protein have helped in localising it inside the cell as well as in studying its presence in some cell cycle (cdc) mutants.

1419 YEAST MUTANT DEFECTIVE IN CELL-CYCLE ARREST. Malcolm Whiteway and Jack Szostak Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Arrest of the mitotic cell-cycle in S. cerevisiae involves the interaction of external signals such as mating pheromones and nutrient levels, with internal signals defining cell type. The ARD1 gene (arrest defective) gene was defined by a spontaneous mutant defective in the normal arrest of the mitotic cell-cycle. Ard1 mutants failed to respond to alpha-factor, and failed to enter stationary phase or sporulate when limited for nutrients. The ARD1 gene has been cloned from a YCp50 clone bank. The ARD1 open reading frame defines a 192aa. protein with no significant homology to any previously identified gene involved in cell-cycle regulation. Insertion of the HIS3 gene into the ARD1 reading frame shows that the ARD1 gene is not essential; this null mutant has the same phenotype as the original mutant. In addition to the defect in cellular arrest, the ard1 mutants are defective in the expression of all tested a-specific genes. Mata cells carrying the ard1::HIS3 mutation mate at 10^{-3} relative to wild-type a cells, make reduced levels of a-factor, fail to agglutinate, do not express Barrier activitiy, and have reduced levels of expression of a ste6-lacZ fusion. Mat alpha cells carrying the ard1::HIS3 mutation mate normally. This suggests that the interaction between environmental signals and cell type may be complex, and does not simply involve a variety of signals triggering a single arrest mechanism.

Cell Cycle: Oncogene Homologues and Meiosis

1420 GENE EXPRESSION IN SPORULATING CELLS OF SACCHAROMYCES CEREVISIAE, Susan Lindquist & Steve Kurtz, The Department of Molecular Genetics & Cell Biology, The University of Chicago, Chicago, Illinois, 60637

When yeast cells are induced to sporulate they undergo a dramatic series of changes in gene expression. Shortly after transfer to nitrogen free medium, mRNAs for certain proteins are repressed while a broad array of messages for other proteins are induced. At distinct times later in sporulation, two sets of messenger RNAs are induced in a/α but not in a/a or α/α cells. (Kurtz, S. and Lindquist, S. (1984) P.N.A.S. (USA) 81: 7323-7327). The first set appears at the time of spore wall deposition. In vitro translation of some of these messages is affected by Signal Recognition Particle. Taken together with the fact that the spore wall is deposited between a double membrane that surrounds the prospore, these findings support the notion that the first set of messages in involved in spore wall synthesis. These messages are concentrated in the ascal cytoplasm, suggesting the spore wall is synthesized by the maternal cytoplasm, not by the spore. The second set of sporulation specific messages appears as the first set is degraded. These messages are produced inside the developing spore and are absent from the ascal cytoplasm. At the time of their production, the transcriptional repertoire of the cell has been dramatically altered: The cells are refractory to induction of hsp70, the major heat shock protein.

1421 ROLE OF CAMP IN CELL DIVISION, Kunihiro Matsumoto[1], Isao Uno[2] and Tatsuo Ishikawa[2], [1]Department of Industrial Chemistry, Tottori-University, Tottori; [2]Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan

In an attempt to determine the role of cAMP in yeast, we isolated three groups of cAMP-requiring mutants, cyr1, cyr2 and CYR3 (1). The cyr1 mutation caused the deficiency of adenylate cyclase (2). The cyr2 mutation was altered in the catalytic subunit of cAMP-dependent protein kinase. The CYR3 mutation caused the alteration of the regulatory subunit of cAMP-dependent protein kinase which had high Ka values for cAMP (3). The cyr1, cyr2 and CYR3 mutations were suppressed by the bcy1 mutation, which resulted in deficiency of the regulatory subunit of cAMP-dependent protein kinase and production of high level of cAMP-independent protein kinase (1). The cyr2 mutation was suppressed by the ppd1 mutation, which was defective in phosphoprotein phosphatase. The CYR3 mutation was suppressed by the pde1 mutation, which was deficient in high Km phosphodiesterase (4). The cyr1, cyr2 and CYR3 mutants were arrested at the G1 phase of the mitotic cell cycle in the absence of cAMP. The phenotype of these cyr mutants mimicked those of nutritionally limited cells. The bcy1 and ppd1 mutations suppressed G1 arrest caused by nutritional limitation. The temperature-sensitive (ts) cyr1, cyr2 and CYR3 mutations permitted the initiation of meiosis, but resulted in the frequent production of two-spored asci at the restrictive temperature. Unlike the wild type cells, these ts mutants cells were capable of initiating meiosis in nutrient growth medium. In diploids homozygous for the bcy1 or ppd1 mutations, no premeiotic DNA replication occurred, and no spores were formed. These data suggest that cAMP works as a positive effector at the start of yeast mitosis via the activation of cAMP-dependent protein kinase, but that it does as a negative effector at the start of meiosis.

(1) K. Matsumoto et al. Proc. Natl. Acad. Sci. U.S.A. 79: 2355-2359 (1982)
(2) K. Matsumoto et al. J. Bacteriol. 157: 277-282 (1984)
(3) I. Uno et al. J. Biol. Chem. 257: 14110-14115 (1982)
(4) I. Uno et al. J. Biol. Chem. 258: 3539-3542 (1983)

1422 **YEAST RAS GENES ARE REQUIRED FOR PROPER NUTRIENT RESPONSE.**
K.Tatchell*, L.Robinson*, J.Cannon*, R.Schultz*, M.Breitenbach+, D.DeFeo-Jones,# G. Temeles#, J. Gibbs#, I.Sigal# and E.M.Scolnick#. *Dept. of Biology, University of Pennsylvania, Philadelphia, PA 19104. +Institut fur Allgemeine Biochemie, Wahringerstrasse 38, 1090 Vienna, Austria. #Dept. of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Saccharomyces cerevisiae contains three unlinked genes which share homology with the ras oncogene family: YP2[1], RAS1[2], and RAS2[2]. The RAS1 and RAS2 gene products can be immunoprecipitated by antibodies against mammalian ras[3] and hydrolyze GTP. We report here that yeast RAS proteins containing missense mutations at amino acid positions known to increase transformation in mammalian cells have decreased intrinsic GTPase activity.

Spores containing gene disruptions of both RAS1 and RAS2 fail to grow but cells containing single RAS disruptions are viable[4,5]. Although neither gene is absolutely essential, ras2 disruptions have a number of pleiotropic phenotypes which include: 1) the inability to effectively utilize non-fermentable carbon sources, 2) the hyperaccumulation of the storage carbohydrates glycogen and trehalose and, 3) the ability to sporulate on rich media. These phenotypes suggest that decreased levels of RAS lead to premature starvation response.

We have isolated extragenic suppressors of the respiratory defect of ras2 which fall into a number of complementation groups. Alleles in two of these bypass the requirement for RAS, allowing cells containing neither RAS gene to grow. One of these mutations, sra1, has a pleiotropic phenotype consistent with loss of ability to respond to starvation signals. Similar phenotypes are observed in yeast cells which contain a $RAS2^{Val19}$ missense mutation. However another bypass mutant (sra3) has none of these phenotypes. The phenotypes of these mutants and observations of altered levels of cAMP and membrane bound adenlyate cyclase activity in some of these mutants suggests that RAS is required for proper nutrient response.

[1]Gallwitz,D.,Donath,C., & Sander,C. Nature 306, 704-707.(1983)
[2]DeFeo-Jones,D. et al. Nature 306, 707-709.(1983)
[3]Papageorge,A.G. et al. Molec. Cell. Biol. 4, 23-29.(1984)
[4]Kataoka,T. et al. Cell 37, 437-445.(1984)
[5]Tatchell,K. et al. Nature 309, 523-527.(1984)

Cytoskeleton and Organelles

1423 GENETICS OF VACUOLAR PROTEASES, E. Jones, C. Moehle, M. Kolodny, M. Aynardi, F. Park, L. Daniels, Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213.

Plasmids capable of complementing the prb1-1122 nonsense mutation were recovered from the Carlson and Botstein YEp24 bank, and the Nasmyth and Reed YEp13 bank. That the complementing plasmids each contained the PRB1 gene was confirmed by segregational analysis of integrant crosses. From the same YEp13 bank many different inserts capable of complementing the pep4-3 mutation were isolated. Of seven examined in some detail, none proved to contain the PEP4 gene. The PEP4 gene was recovered from the YEp24 bank by complementation. Proof of its recovery was based on segregational analyses of crosses involving integrants.

Strains were constructed which carried a YEp (PRB1) plasmid, a YEp (PEP4) plasmid, both plasmids or no plasmid and with a wild type chromosomal genotype for protease genes. Expression of protease B was followed during growth of these strains. In the plasmid-free strain, the levels of protease B rose at least 10 fold as the cells approached stationary phase, similar to results previously reported (1). In cells containing the PEP4 bearing plasmid, the level of derepression was comparable to that seen in the absence of the plasmid but derepression occurred much earlier in the growth curve. This finding is not unexpected since pep4-3 heterozygotes show a dosage effect on protease B levels (2). Cells bearing the PRB1 containing plasmid derepress levels of protease B more than 40 fold and the derepression occurs as the cells enter the diauxic plateau. Cells bearing both plasmids derepress protease B at the diauxic plateau and beyond. Derepression is at least 100 fold. The effects of the PRB1 and PEP4 plasmids appear to be multiplicative. The significance of this relationship is unclear, since the timing of derepression as well as the extent of derepression are affected. Late in the growth curve of the strain bearing both plasmids, the specific activity of protease B in cells falls. Concomitantly, substantial amounts of active protease B are secreted into the medium.

1) Saheki, T., Holzer, H. 1975. Biochim. Biophys. Acta 384: 203-214.
2) Zubenko, G. 1981. Ph.D. thesis, Carnegie-Mellon University.

1424 GENES CONTROLLING MORPHOGENETIC STEPS OF THE CELL CYCLE, J.R. Pringle, S. Lillie, B. Haarer, K. Coleman, J. Robinson, A. Stapleton, A. Adams, R. Preston, and C. Jacobs, Divis. Biological Sciences, University of Michigan, Ann Arbor, MI 48109

We are engaged in a genetic analysis of morphogenesis during the cell cycle of the yeast Saccharomyces cerevisiae. We are isolating and characterizing temperature-conditional mutants with defects in morphogenesis, isolating and characterizing extragenic suppressors of these morphogenetic mutations, and cloning the genes identified by the morphogenetic and suppressor mutations as a means to identify and characterize the gene products. Recent progress includes the identification of several new complementation groups of cold-sensitive mutants that produce multiple, abnormally elongated buds at restrictive temperature. Also, we have isolated and analyzed numerous extragenic suppressors of cdc3, cdc10, cdc11, cdc12, cdc24, and cdc42 mutations. Interestingly, most of the suppressors are recessive to their wild-type alleles and appear to yield some unexpected "intergenic noncomplementation". Also, we have found that mutations at CDC3 can suppress mutations at CDC10, and vice versa, presumably reflecting a direct interaction between these gene products. Finally, we have cloned the CDC3, CDC10, CDC11, CDC12, and CDC24 genes and have begun attempts to identify the products of these genes. Interestingly, plasmids carrying CDC12 can complement cdc11 mutations as well as cdc12 mutations, although the former complementation appears to require a chromosomal change in the recipient strain (disomy for the cdc11-caarying chromosome?) as well as the presence of the CDC12-carrying plasmid. Fusions of CDC24 to the E. coli lacZ gene and of CDC12 to the E. coli lacZ and trpE genes have been constructed and, in the latter case, the resulting fusion proteins have been used to generate antisera that are now being characterized. The hope is that these antisera (and those yet to be raised against similar fusion proteins for the other genes of interest) will allow us to localize and begin to characterize the functions of the gene products of interest. Some of these results have been summarized elsewhere (1).

1. Pringle, J.R., Coleman, K., Adams, A., Lillie, S., Haarer, B., Jacobs, C., Robinson, J., and Evans, C. (1984) "Cellular morphogenesis in the yeast cell cycle", In Molecular Biology of the Cytoskeleton (G. Borisy, D. Cleveland, and D. Murphy, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor. In press.

1425 NUCLEAR FUSION IN YEAST, Mark D. Rose and Gerald R. Fink, Whitehead Institute for Biomedical Research and Department of Biology, MIT, Cambridge, MA 02142

During conjugation, two haploid nuclei fuse to form a diploid nucleus. Nuclear fusion is not a passive process and is mediated by extranuclear microtubules extending from the spindle plaque. Several mutations have been isolated that disrupt nuclear fusion while not otherwise affecting conjugation. We have been examining these mutations with the aim of identifying the proteins and structures responsible for this nuclear function.

The kar loci (for karyogamy defective) define genes that are required for nuclear fusion. Kar$^-$ mutations at these loci are recessive, yet only one parent need be mutant for fusion to fail. Moreover, nuclei in the heterokaryotic zygotes fail to fuse during subsequent mitotic division. We present evidence that nuclei must be activated for nuclear fusion prior to conjugation and activation is dependent on both the KAR1 gene product and mating hormones.

We have isolated the KAR1 gene by complementation of the kar1-1 mutation. The KAR1 gene is toxic in high copy number. Replacement of the single chromosomal KAR1 gene with null alleles constructed in vitro results in a recessive lethal phenotype. KAR1 is essential for viability of both haploids and diploids.

The transcript of the KAR1 is regulated by the mating type locus. Homozygosity at MAT results in higher levels of expression whereas heterozygosity results in lower levels. After treatment of MATa haploids with α factor, transcript levels are reduced to the level of a/α diploids, suggesting that high levels of activity are required only prior to but not during mating.

Examination of cytoskeletal elements in shmoos and zygotes reveals a specific defect in extranuclear microtubule structure in kar1-1 cells. Upon treatment with α factor, the mutants elaborate an aberrantly long extranuclear microtubule structure. This aberrant structure, made prior to mating, could prevent nuclear fusion either by steric hindrance or by the absence of an essential structure.

We have been using a spheroplast fusion assay to examine nuclear fusion independently of conjugation. Fusion is stimulated 1000 X by prior treatment with α factor. Stimulated fusion is dependent on KAR1 in a manner similar to mating. As early as one can fuse stimulated cells, the KAR1 gene product is not able to complement the mutant nucleus in trans, indicating the KAR1 dependent step has been passed.

Finally, antibodies to the KAR1 gene product have been produced. Immunoflourescence has been used to localize the KAR1 product in mating cells.

1426 Molecular Analysis of Cytoskeleton. Lorraine Pillus and Frank Solomon, Department of Biology, M.I.T., Cambridge, MA 02139

As part of a study of microtubule structure and function, we have begun an analysis of yeast microtubule proteins. The approach we take to identify these proteins is based on methods we have applied to cultured cells. In those experiments, cells are extracted with non-ionic detergents so that 70 per cent of the total protein is released under conditions that preserve cellular microtubules. These structures are then quantitatively depolymerized, and their components solubilized by a second extraction including calcium ions. The microtubule components can be identified by comparing calcium extracts from cells with and without microtubules. The components of different microtubule structures which arise in different cell states can also be identified and the assembled and unassembled pools of components can be compared. This approach enables one to work with very small amounts of material, and to analyze microtubules as they exist in vivo rather than to rely on in vitro reconstitution techniques. The basic features of this assay - extraction under stabilization conditions and depolymerization of microtubules both in vivo and in vitro - are now in hand for S. cerevisiae. The morphological correlate of each state has been demonstrated. We are now using the assay to identify microtubule components in yeast. The goal of this work is to use molecular and genetic analysis in concert to understand microtubule functions.

Regulation and Recombination

1427 **INTERACTION OF THE FLP RECOMBINASE WITH SUBSTRATE 2-MICRON CIRCLE DNA**
Brenda J. Andrews, Linda Beatty and Paul D. Sadowski, U. of Toronto

The yeast plasmid 2-micron circle encodes a site-specific recombinase designated FLP which promotes efficient inversion across two precise 599 base-pair inverted repeat sequences on the 2-micron molecule. We purified the FLP protein from E. coli cells transformed with a plasmid containing FLP coding sequences transcribed from an efficient promoter. Using purified enzyme preparations, we undertook nuclease protection and filter binding experiments to study the sites within the inverted repeat sequences that are important for binding of FLP to substrate DNA. We find that FLP protects about 50 base-pairs of DNA from non-specific nuclease digestion. The protected site consists of two 13 base-pair inverted repeat sequences separated by an 8 base-pair spacer region. A third 13 base-pair element is also protected by binding of the FLP protein. We find that FLP promotes site-specific cleavage of substrate DNA within the 8 base-pair spacer region. We have looked at these FLP-DNA interactions on intact ('wild-type') substrate and various mutant substrates. We discuss the structural features of the binding site with respect to the mechanism of FLP-mediated recombination.

1428 SUPERCOILING, CHROMATIN STRUCTURE, AND THE REGULATION OF ACID PHOSPHATASE GENE EXPRESSION, Lawrence W. Bergman, Martin C. Stranathan, Connie S. Agnor and Lisa Preis, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228.

To investigate the mechanism of transcriptional activation, we have developed a high copy plasmid system containing the entire structural and regulatory sequences of the acid phosphatase (PHO5) gene and the TRP1/ARS1 sequences of the yeast Saccharomyces cerevisiae. The resulting 3.4kb plasmid has been utilized to transform either wild type or a number of acid phosphatase regulatory mutant strains. Analysis of mRNA levels under repressed and derepressed growth conditions reveals that normal transcriptional regulation of the gene persists, although gene copy number has been increased approximately 50-fold. Analysis of changes in the linking number of the plasmids isolated under repressed and derepressed growth conditions has revealed that the transcriptionally active plasmid contains 2-3 less negative supercoils than the inactive plasmid. This difference in linking number is similarly seen in a plasmid containing a second sequence-related phosphate-repressible acid phosphatase gene, however, is not seen in plasmids isolated from either fully constitutive or non-derepressible regulatory mutants suggesting that the change in linking number is associated with the mechanism of regulation of the acid phosphatase gene. Finally, analysis of the chromatin structure of these plasmids has revealed a localized disruption of nucleosome positioning associated with transcriptional activity.

1429 STRUCTURE-FUNCTION STUDIES OF THE YEAST GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE GENE PROMOTER. Grant A. Bitter and Kevin M. Egan, Amgen, Thousand Oaks, CA 91320

Heterologous proteins have been expressed in Saccharomyces cerevisiae from vectors utilizing the glyceraldehyde-3-phosphate dehydrogenase gene (GPD) portable promoter [G. A. Bitter and Egan K. M. (1984), Gene, in press]. The level of heterologous protein expression varies depending on the heterologous gene cloned in the vector, demonstrating that DNA sequences in addition to the promoter and transcription terminator determine expression levels. By optimizing DNA sequences around the translation initiation site of the HBsAg gene, a ten- to fifteen-fold increase in expression level was observed. The effects of codon utilization on translation elongation have also been investigated.

Deletion of nucleotides -676 to -380 of the GPD portable promoter results in a ten-fold decrease in heterologous protein expression levels, indicating a role of these sequences in promoter function. A unique SalI site was engineered 240 bp 5' to the TATA box of the GPD portable promoter. Upstream regulatory sequences from other yeast genes have been cloned into this site and the effect on GPD promoter function quantitated.

1430 THE ISOLATION OF ALCOHOL OXIDASE AND TWO OTHER METHANOL REGULATABLE GENES FROM THE YEAST, PICHIA PASTORIS, Paul F. Brust, Steven B. Ellis, Patricia J. Koutz, Ann F. Waters, Michael M. Harpold and Thomas R. Gingeras, Salk Institute Biotechnology/Industrial Associates, Inc., La Jolla CA 92037

The oxidation of methanol follows a well defined pathway and is similar for several methanotrophic yeasts. The use of methanol as the sole carbon source for the growth of P. pastoris stimulates the expression of a family of genes. Three methanol responsive genes have been isolated, cDNA copies made from the mRNAs of these genes and the protein products from in vitro translations examined. The identification of alcohol oxidase as one of the cloned methanol-regulated genes has been made by enzymatic, immunological and sequence analyses. Methanol regulated expression of each of these three isolated genes can be demonstrated to occur at the level of transcription. Finally, DNA subfragments of two of the methanol responsive genomic clones from P. pastoris have been isolated and identified as containing the control regions involved in methanol regulation.

1431 DESCRIPTION OF PICHIA PASTORIS AS A HOST SYSTEM FOR DNA TRANSFORMATIONS, J. M. Cregg, K. J. Barringer, A. Y. Hessler, and K. R. Madden, SIBIA, La Jolla, CA 92037.

Methanol utilizing yeast are of interest in understanding the regulation of gene expression by methanol and in determining the mechanism by which enzymes such as the alcohol oxidase of methylotrophs are compartmentalized in peroxisomes. These studies would be greatly facilitated by the ability to transform the yeast.

We have developed the methylotrophic yeast, Pichia pastoris, as a host system for DNA transformations. The system is based on a histidinol dehydrogenase defective mutant of P. pastoris (his4) and the spheroplast generation-$CaCl_2$ PEG fusion procedure. As a selectable marker, the P. pastoris HIS4 gene has been isolated from a P. pastoris-YEp13 library by complementation of a Saccharomyces cerevisiae his4ABC- host. Plasmids which contain either a Pichia or Saccharomyces HIS4 gene transform the P. pastoris his4- host to histidine prototrophy.

Initially, any plasmid which contained DNA sequences homologous to the Pichia genome integrated into the genome. Autonomous replication sequences (ARSs) from S. cerevisiae which we examined did not maintain plasmids as autonomous elements in P. pastoris. Therefore, DNA fragments with ARS activity in Pichia were selected from a P. pastoris DNA library by transformation into P. pastoris. Two fragments, PARS1 and PARS2 were isolated which increase transformation frequencies of plasmids as much as 10^4 fold to about $1x10^5/\mu g$ and maintain plasmids as autonomous elements in P. pastoris for at least 50 generations in selective medium, even when the plasmids contain long sequences homologous to the Pichia genome.

1432 ANALYSIS OF A NONTRANSCRIBED REGION 5′ TO A RIBOSOMAL PROTEIN GENE. David M. Donovan and Nancy J. Pearson, University of Maryland, Catonsville, MD 21228.

Ribosomal protein genes (rp) in yeast may be regulated at the transcriptional and translational level. We have sequenced a linked pair of rp genes (rp28-rp55 copy 1) and duplicate copies of these genes which exist in a similar linkage arrangement (rp28-rp55 copy 2) in the genome. We are analyzing the intergenic region which is 5′ to rp55 transcript to determine how this region is involved in transcriptional regulation of the rp55 gene. Some of the short sequence homologies found 5′ to other yeast rp genes are also present in these intergenic regions upstream from copy 1 and copy 2 of rp55. However, the exact position of these short homologies is not conserved between duplicate copies. We have used two approaches thus far to examine this 5′ nontranscribed region. First we have attempted to correlate DNaseI sensitive sites 5′ to rp55 with the location of the short rp gene sequence homologies present in this region. Secondly, we have constructed a fusion between the nontranscribed intergenic region 5′ to rp55 and a previously characterized cycl-lacz fusion (PNAS 78:2199). Since this fusion contains none of the transcript of rp55 (which could contribute to translational control of this gene) we can determine whether this upstream nontranscribed region alone is sufficient to regulate β-galactosidase expression as a ribosomal protein under conditions where ribosomal proteins are assumed to be transcriptionally regulated.

1433 A DIHYDROFOLATE REDUCTASE GENE FROM YEAST. Christopher G. Goff and Barri Fessler, Haverford College, Haverford, PA, 19041

S. cerevisiae apparently has two distinct isozymes of dihydrofolate reductase (E.C. 1.5.1.3): one a soluble cytoplasmic enzyme (a monomeric protein 27,000 daltons in MW; Goff, unpublished), accounting for ~95% of the dihydrofolate reductase activity in the cell; and the other (of uncertain MW) embedded in the mitochondrial membrane, exhibiting ~5% of the total activity (Zelikson and Luzzati, E.J.B. 79, 285). Mutations in a nuclear gene, tmp3, eliminate the mitochondrial enzyme's activity (along with the activity of several other enzymes of mitochondrial folate metabolism) and are (conditionally) lethal despite the continued presence of the cytoplasmic isozymes (Zelikson and Luzzati, op. cit.). No mutations affecting the cytoplasmic isozyme have been reported. We have obtained from Dr. Barry Barclay (Toronto) a cloned yeast DNA fragment, isolated from the Friesen yeast bank, which appears to code for the cytoplasmic dihydrofolate reductase. A 1.6 kb BamHI - SalI yeast fragment, cloned into pBR313, confers trimethoprim resistance on E. coli cells which carry it. E. coli with the plasmid produces a small amount of soluble 27,000 dalton dihydrofolate reductase activity (in addition to the ~15,000 dalton E. coli enzyme), which is sensitive to aminopterin but resistant to trimethoprim, as expected for the yeast enzyme. We have generated a restriction map of this DNA fragment and are now 1) sequencing it to compare the polypeptide to other characterized dihydrofolate reductases, 2) integrating it on shuttle vectors to identify the chromosome carrying the gene, and 3) creating mutants in it by "gene disruption". We intend eventually to determine the structural and genetic relationship between the cytoplasmic and mitochondrial enzymes.

1434 **A NOVEL METHOD TO DETERMINE DNA SEQUENCES ESSENTIAL FOR FLP-MEDIATED RECOMBINATION** **Richard M. Gronostajski, Paul D. Sadowski, Univ. of Toronto, Toronto, Ont., M5S 1A8**

The yeast 2 micron plasmid encodes a protein, FLP, that mediates site-specific recombination. When two FLP sites are present on a substrate in a direct orientation, FLP-mediated recombination excises the DNA from between the sites. We have used a novel technique, 'Nuclease-Primed Substrate Analysis', to determine the minimal duplex DNA sequence needed for this recombination event. A linear DNA with two FLP sites in a direct orientation is treated with a double-strand specific 3'-exonuclease (ExoIII) to generate molecules with a nested set of single-strand deletions that extend into one of the FLP sites. The DNA is then end-labeled at the sites of the deletions using an α-^{32}P labeled dNTP and Klenow polymerase. Cleavage of the DNA at a restriction endonuclease site present between the two FLP sites produces a ladder of labeled fragments extending from the cleavage site to the deletion at the 3' end of each molecule. If the DNA is treated with FLP prior to endonuclease digestion, molecules containing two intact FLP sites will undergo intramolecular recombination and excise the restriction cleavage site from between the FLP sites. Endonuclease digestion of this FLP-treated DNA generates labeled fragments only from molecules that were deleted beyond the minimal DNA sequence needed for FLP activity. Comparison of the fragments produced by restriction enzyme digestion of untreated and FLP-treated DNA shows to the nucleotide the DNA sequence required for FLP-mediated recombination. A modified form of this technique can be used to study the minimal sequence requirements of site-specific DNA binding proteins.

1435 MEIOTIC RECOMBINATION BETWEEN REPEATED SEQUENCES ON NONHOMOLOGOUS CHROMOSOMES IN S. CEREVISIAE, Sue Jinks-Robertson and Thomas D. Petes, University of Chicago, Chicago, IL 60637

The yeast transformation system was used to construct a diploid strain with different selectable markers (URA3$^+$ and HIS3$^+$ genes) at identical positions on chromosome IX homologs. The meiotic segregation of the artificially-constructed alleles was examined by tetrad dissection and, as expected, most spores had either the HIS3$^+$ or URA3$^+$ gene. At a frequency of about 0.5%, however, tetrads were found containing a spore with neither of the wild type genes. Physical analyses of the aberrant tetrads demonstrated that the His$^-$Ura$^-$ spores were the result of a nonreciprocal recombination (gene conversion) event involving the HIS3$^+$ gene inserted into chromosome IX and a His3$^-$ allele at the HIS3 locus on chromosome XV. The meiotic frequency of gene conversion between the repeated HIS3 genes on nonhomologous chromosomes was similar to that observed between allelic sequences at the HIS3 locus.

The high frequency of conversion between the repeated HIS3 genes on nonhomologous chromosomes questions the presumed mechanistic relationship between gene conversion and reciprocal recombination, since reciprocal exchanges accompanying gene conversions between naturally-occurring, dispersed repeats would result in numerous chromosomes aberrations. Experiments are in progress which will determine whether conversion events between nonhomologous chromosomes are associated with reciprocal exchanges.

1436 GENETICS AND BREEDING OF LAGER YEAST, M.C. Kielland-Brandt, C. Gjermansen, S. Holmberg, T. Nilsson-Tillgren**, M.B. Pedersen*, J.G.L. Petersen, J. Polaina and P. Sigsgaard* Carlsberg Laboratory and *Carlsberg Research Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby and **Institute of Genetics, University of Copenhagen, Denmark

Yeast strains used in lager beer production are closely related. With several probes, including Ty, their Southerns are virtually identical, as opposed to the variation seen when different Saccharomyces species are compared. Until recently, their genetics were obscure due to low spore viability. However, internuclear chromosome transfer mediated by the karl mutation has enabled us to study single chromosomes of lager yeast in the genetic background of strains with normal marker segregation. In connection with Southern hybridization analysis this technique has revealed extensive structural heterozygosis, resembling species hybrids in plants. This intrinsic genetic variation is utilized in a promising cross breeding program.

A specific aim for strain improvement is the reduction of diacetyl production. This off-flavor compound is formed in a side reaction from an intermediate in the biosynthesis of valine. We have therefore undertaken a study of the valine and isoleucine pathways in academic yeast. The genes known for the enzymes in these pathways have been mapped, cloned and partially sequenced. Synthesis of the enzymes is regulated in response to two kinds of stimuli: Starvation for any amino acid causes increased synthesis (general control) of two of the enzymes while the simultaneous presence of isoleucine, valine and leucine results in repression (multivalent repression) of all of them. These responses are being correlated with the corresponding mRNA levels and presence of promoter signals.

1437 CLONING OF cDNAs CODING FOR CATALASE AND OTHER PEROXISOMAL PROTEINS OF CANDIDA TROPICALIS, Paul B. Lazarow, Richard A. Rachubinski and Yukio Fujiki, The Rockefeller University, New York, NY 10021

The biogenesis of all animal and plant peroxisomal proteins investigated to date begins on free polyribosomes and almost all of them (including a major integral membrane protein, PNAS 81:7127, 1984) are synthesized at their final sizes. We have undertaken the cloning of cDNAs coding for peroxisomal proteins in order to obtain detailed structural information that may be relevant to their intracellular transport. Candida tropicalis was grown on alkanes, which causes the proliferation of peroxisomes and the induction of peroxisomal enzymes including catalase and β-oxidation enzymes, or on glucose, in which few peroxisomes form. A cDNA library was constructed complementary to mRNAs of alkane-grown cells. The library was screened for sequences induced by growth on alkanes by differential DNA dot blot hybridization using [^{32}P]cDNA complementary to poly(A$^+$)RNA of alkane- or glucose-grown C. tropicalis. Positive clones (9% of the total) were subjected to hybridization-selection translation. Seven clones have been identified that hybridize to mRNAs whose cell-free translation products comigrate with polypeptides of highly purified C. tropicalis peroxisomes. Among these, clones coding for catalase and for acyl-CoA oxidase have been positively identified by immunoprecipitation of the translation products with specific antisera. RNA dot blots and cell-free translations confirm that growth of C. tropicalis on alkanes causes striking increases in the expression of mRNAs coding for these two enzymes.

1438 **TRANSLATIONAL REGULATION OF THE ACTIVATOR OF GENERAL AMINO ACID CONTROL IN YEAST, Peter P. Müller and Alan G. Hinnebusch, NIH, Bethesda, MD 20205**

The GCN4 gene encodes a trans-acting positive regulator of amino acid biosynthetic genes in S. cerevisiae. The expression of this activator is itself controlled by trans-acting factors that respond to amino acid levels and this regulation occurs at the translational level. The GCN2 and the GCN3 products act as positive regulators of GCN4 translation during amino acid starvation, whereas the GCD1 product appears to act as a translational repressor. The GCN4 mRNA 5' leader is nearly 600 nucleotides in length and contains four small open reading frames, each of which is two or three codons in length and complete with initiation and termination codons. A 250 bp deletion of the small open reading frames leads to constitutive derepression of GCN4 translation. We are investigating the hypothesis that the small open reading frames in the GCN4 mRNA 5' leader are in fact the cis-acting sequences that repress translation initiation at the GCN4 coding sequences. A mutation in the GCD1 gene also leads to constitutive derepression of GCN4 expression, suggesting that GCD1 mediates the translational repression exerted by the GCN4 mRNA 5' leader sequences. Either of these mutations suppresses the requirement of the GCN2 and GCN3 products, suggesting that GCN2 and GCN3 antagonize these negative regulatory elements in response to amino acid starvation.

1439 Characterization of MOD5, A Gene Essential for Modification of Both Mitochondrial and Cytoplasmic tRNA in Saccharomyces cerevisiae. D.R. Najarian, M. Dihanich, A. Hopper and N.C. Martin, University of Texas Health Science Center at Dallas, Dallas, TX 75235, Dept. of Penn. State Univ., Hershey, PA 17033.

Based on the biochemical and immunological non-identity of proteins with analogous functions in different cellular compartments, it has been hypothesized that such proteins must be encoded by separate nuclear genes. However, the mod5-1 mutation of S. cerevisiae is a single nuclear mutation which simultaneously abolishes the isopentenyl modification of both cytoplasmic and mitochondrial transfer RNA. A 7.0 kb yeast genomic fragment was cloned by complementation of the mod5-1 loss of suppression phenotype, and shown genetically to be an allele of MOD5. Mutant cells transformed with YEpMOD5 (1.6), a multicopy plasmid carrying a 1.6 kb fragment subcloned from the original isolate, are restored with respect to isopentenylation of both mitochondrial and cytoplasmic tRNA. In vitro measurements of the transfer of the isopentenyl group of Δ^2 isopentenyl pyrophosphate to tRNA detect elevated activity with respect to wild-type in extracts of YEpMOD5 (1.6) transformants, but no activity in mutant cell extracts. Incomplete DNA sequence information reveals a large open reading frame. Analysis of the DNA sequence and RNA and protein products of MOD5 are underway and should answer the question of whether different forms of the transferase are encoded by the same genetic information or whether identical proteins are found in both compartments. (Supported by NSF Grant PCM830221).

1440 ANALYSIS OF THE RAD3 GENE OF SACCHAROMYCES CEREVISIAE, Louie Naumovski and Errol C. Friedberg, Dept. of Pathology, Stanford Medical School, Stanford CA 94305

The RAD3 gene of S. cerevisiae is required for the excision repair of DNA damaged by UV-light and certain chemicals. Previously existing mutations in the RAD3 gene had rendered cells sensitive to DNA damage but did not affect viability. However, we have shown by disruption experiments with the cloned gene that it is also necessary for cell viability under normal conditions. We have sequenced the gene and found an open reading frame of 778 aa that could encode a protein of 90kDa. We have also isolated several UV sensitive viable chromosomal alleles by gap repair of centromeric plasmids and have sequenced these to map the mutations. Additionally, we have constructed several mutant alleles by in vitro mutagenesis.

Since RAD3 temperature sensitive mutants are not available, we have devised an approach to examine the nature of the essential function based on regulatable expression of a plasmid borne RAD3 gene in cells with a chromosomal gene disruption (rad3^0). The RAD3 gene was placed under control of the GAL1 promoter. UV sensitive rad3 cells transformed with this plasmid are still UV sensitive when grown on dextrose but are UV resistant when grown on galactose, an inducer of the GAL1 gene. The plasmid was introduced into cells carrying a disruption of the chromosomal gene by first transforming a diploid (rad3/rad3^0) and then dissecting tetrads on galactose-containing medium. These cells grow well on galactose but when transferred to dextrose, growth slows after 24hr and eventually ceases. During this period the cellular and the budding morphology is altered. Further studies using this strain construction may be helpful in elucidating the nature of the essential function of RAD3.

1441 The Nucleotide Sequence of the RAD2 Gene from S. cerevisiae, Charles M. Nicolet, Janet Chenevert, and Errol C. Friedberg, Dept. of Pathology, Stanford University, Stanford, CA 94305

RAD2 is one of several genes required in Saccharomyces cerevisiae for excision repair of DNA damage; specifically, RAD2 is involved in the early stage of incision near the site of damage. In an effort to understand the molecular mechanism of RAD2 action, the gene was cloned in the lab with the goal of producing enough protein for biochemical analysis. We are currently investigating structural features of the gene. Here we report the sequencing of a 3.9 kilobase region of the chromosome carrying the RAD2 gene. We located an open reading frame of 2928 nucleotides, potentially coding for a 976 amino acid protein with a calculated molecular weight of 111.1 kilodaltons. The nucleotide and amino acid sequences were compared with those of RAD1 and RAD3. Consensus nucleotide sequences among the RAD genes were located in the 5' and 3' noncoding regions, though no striking amino acid homologies were detected.

1442 THE EFFECT OF micRNA ON GENE EXPRESSION IN YEAST, Ophry Pines and Masayori Inouye, Department of Biochemistry, SUNY at Stony Brook, Stony Brook, NY 11794

Recently, a number of publications have established the ability of antisense transcripts (termed micRNA) to regulate gene expression in prokaryotic cells. The yeast Saccharomyces cerevisiae was chosen to test the existence and mechanism of this new type regulation in eucaryotic cells due to advanced techniques in molecular biology and genetics of this system. Fragments from two genes coding for actin and the iso-1-cytochrome C of yeast were subcloned into plasmid shuttle vectors capable of replicating both in E. coli and yeast. The cloned fragments from these genes were inserted downstream to an inducible yeast promoter in reverse orientation to the natural genes. When yeast transformant cells carrying the above plasmids were appropriately induced, antisense transcripts of yeast actin and cytochrome C were produced. Currently experiments are in progress to determine the effect of such plasmid constructions on transcription, translation, and phenotypic expression of actin and cytochrome C. RNA from yeast cells has been isolated and tested with various probes to determine the relative amounts of antisense transcripts from the plasmids, natural transcripts from the affected chromosomal genes, and transcripts from unrelated genes. Fusions between the coding sequences of yeast genes and the E. coli lacZ gene are being used to determine the relative amount of translation of such genes in the presence or absence of antisense mRNA. Experiments to determine the effect of the micRNA genes on the yeast growth and cell cycle are in progress.

1443 REGULATION OF YEAST GENES INDUCED BY DNA DAMAGING AGENTS, Stephanie W. Ruby[1,2] and Jack W. Szostak[2], [1]Dept. of Biological Chemistry, Harvard Medical School and Dept. of Molecular Biology, Mass. General Hospital, Boston, MA 02114.

The yeast Saccharomyces cerevisiae has a set of genes that are induced in response to DNA damaging agents. We have previously identified and cloned six DNA damage inducible (din) genes as fusions to the E. coli lacZ gene. The expression of each fusion is controlled by the promotor of the yeast gene. Currently we are studying the regulation of the din genes by identifying unlinked regulatory genes. We have cloned one gene that affects the expression of one din gene but not of another one. This trans-acting gene (dex for damage expression) raises both the basal and damage induced levels of the DIN1 transcript. Experiments further defining the role of DEX in the regulation of DIN1 will be presented.

1444 HOMOTHALLIC SWITCHING AND GENE CONVERSION IN YEAST, Jeffrey Strathern[1], Margaret Kelly[2], Brenda Shafer[1], and Carolyn McGill. [1]NCI-Frederick Cancer Cancer Research Facility, Laboratory of Eukaryotic Gene Expression, P.O. Box B, Frederick, MD 21701; [2]Cold Spring Harbor Laboratory, NY 11724.

An unexpressed copy of MATα is stored at the HMLα locus on the left arm of chromosome III, and an unexpressed copy of MATa is stored on the right arm of chromosome III at HMRa. HML and HMR are kept silent by trans-acting functions encoded by the MAR/SIR genes. Some yeast strains (homothallic) can change from the α cell type to the a cell type and from a to α. These switches reflect changes from MATα to MATa and MATa to MATα. They occur by substituting copies of HMRa or HMLα into MAT. These specific genome rearrangements occur at high efficiency (over 80%) in particular cells within a clonal pedigree. Homothallic switching has several features in common with gene conversion. We will present experiments testing the relationship of these processes.

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1445 ANALYSIS OF PLASMID RECOMBINATION CATALYZED BY CELL-FREE EXTRACTS OF SACCHAROMYCES CEREVISIAE. Lorraine Symington and Richard Kolodner, Dana-Farber Cancer Institute, Boston, MA 02115

We have recently described an *in vitro* assay system to investigate the molecular mechanisms of genetic recombination in yeast. This assay utilizes yeast cell-free extracts to catalyze recombination events between homologous plasmids containing different mutant alleles of the Tc or *ARG4* genes. Recombination events were detected by transforming product DNA into an *E. coli recA* (*argH*) strain to select for $Tc^r/ARG4^+$ recombinants. The reaction increased the frequency of Tc^r or ARG^+ transformants (recombinants) from 2×10^{-6} to $1\text{-}3 \times 10^{-3}$. The structures of Tc^r plasmids were determined by restriction mapping and were consistent with a recombination reaction involving gene conversion and crossing over. Electrophoretic analysis of product DNA has indicated that a number of novel DNA species are formed during the reaction, these have been analyzed by electron microscopy. Recombination between circular substrates appeared to involve the formation and processing of figure-8 molecules. To analyze resolution of figure-8 molecules, we have constructed plasmids containing artificial Holliday Junctions. These substrates have been used to purify and characterize an activity from crude extracts that cleave Holliday junctions.

1446 Molecular Cloning and Genetic Mapping of the Yeast *RAD10* Gene
William A. Weiss and Errol C. Friedberg
Department of Pathology, Stanford Medical School, Stanford, CA 94305

The *RAD1*, *RAD2*, *RAD3*, *RAD4*, and *RAD10* genes are required for excision repair of DNA damage in yeast. We have isolated two plasmids which exclusively complement the UV sensitivity of *rad10*-1 and *rad10*-2 mutants. One of these plasmids (pNF101) contains the *RAD10* gene since a plasmid carrying this gene integrates uniquely at the *RAD10* chromosomal locus. The other plasmid (pNF100) contains a tRNA gene which apparently suppresses the *rad10*-1 and *rad10*-2 mutations. The two plasmids bear no detectable homology to each other. The insert in pNF101 was subcloned to 1.1 kb, and this fragment was shown to encode a protein of approximately 33,000 daltons when expressed in *E. coli* maxicells. We have deleted this 1.1 kb fragment from the genome of a wild type strain, and have thus demonstrated that *RAD10* is a non-essential gene. The deletion mutants obtained were significantly more UV sensitive than the corresponding *rad10*-1 or *rad10*-2 alleles. Plasmid pNF101 was also used to probe a blot of yeast chromosomes resolved by two-dimensional pulsed field gradient gel electrophoresis. This experiment demonstrates the *RAD10* gene to be on chromosome XIII.

1447 MEIOTIC GENE CONVERSION OF THE TANDEMLY ITERATED CUP1 GENE
J.W. Welch, D.H. Maloney, and S. Fogel. Univ. of Calif. Berkeley, Ca.

In copper resistant yeast strains the CUP1 locus is a tandemly organized structure composed of up to 14 segments each 2.0kb in length. The locus contains no EcoRl sites. Thus, digestion with EcoRl cleaves only external sites and generates DNA fragments hybridizable to ^{32}P labelled probes specific for the repeat unit. Copy number changes in the region are assessed by the fragment's physical length.

Copy number changes in unselected tetrads from a cross of 9X9 repeat units were analyzed by Southern hybridization. Of 202 complete tetrads 17% show copy number changes. Changes were found in single spores or in two spores of individual tetrads. These classes occur with equal frequency. Only one reciprocal exchange was found. To account for copy number changes, we propose a molecular model based on misaligned pairing of homologous segments followed by gene conversion with and without associated exchange.

1448 A GENE DOSAGE SELECTION SYSTEM FOR DETECTING MITOTIC AND MEIOTIC ANEUPLOIDY IN YEAST. Stephen G. Whittaker*, Beth M. Rockmill*, Michael A. Resnick+, and Seymour Fogel*. *Department of Genetics, University of California, Berkeley, CA 94720, U.S.A. +N.I.E.H.S., P.O. Box 12233, Research Triangle Park, North Carolina, 27709.

The test assays gene dosage at two loci on chromosome VIII using a leaky ts allele of ARG4 and copper resistance conferred by CUP1. Extra chromosomes provide simultaneous increased dosage for both genes and gives rise to copper resistant, arginine prototrophic outgrowths (papillae) on selective medium. Spontaneous and chemically-induced mitotic and meiotic aneuploidy may be assayed in the same culture of the tester strain.

During mitotic cell division in diploids, trisomic (2n+1) papillae arise spontaneously at a rate of approximately 10^{-8} events/cell/division. In meiosis, the spontaneous rate of disome (n+1) production is approximately 10^{-5} to 10^{-6} events/meiosis.

Methylbenzimidazole-2-yl carbamate (MBC) elicits high levels of mitotic and meiotic aneuploidy. Similarly, parafluorophenylalanine (PFA) induces a significant increase over the spontaneous meiotic rate.

The system represents: a) a novel model for examining chromosome behaviour during mitosis and meiosis and b) an extremely sensitive and simple method for examining aneuploidy induction by industrial and environmental chemicals.

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1449 **ISOLATION OF GENES AFFECTING tRNA GENE EXPRESSION IN S. CEREVISIAE AND S. POMBE.** Ian Willis, Vanessa Chisholm and Dieter Söll, Yale University, New Haven, CT 06511.

The tRNA base insertion mutant Ai47:8 derived from the S. pombe sup9-e opal suppressor is suppressor-inactive in single copy. However, on the multi-copy plasmid vector YRp17, this mutant tRNA gene, after transformation into S. cerevisiae strain YH-D5 his4-260(UGA), leu2-2(UGA), trp1-1(UAG) permits weak prototrophic growth. Northern analysis of RNA from these transformants reveals that the biosynthesis of the mutant tRNA is almost but not completely blocked at the first step in splicing - endonucleolytic cleavage (1). Using the YRp17/Ai47:8 clone as a vector we have inserted randomly generated fragments of either S. cerevisiae or S. pombe chromosomal DNA and have selected transformants in S. cerevisiae which exhibit enhanced rates of prototrophic growth. Data will be presented concerning the analysis of the plasmid DNAs recovered from these cells. One approach which has been employed relies on the inability of the S. cerevisiae splicing endonuclease to excise the intron from the S. pombe sup8· leucine tRNA precursor (2). Thus, an S. pombe DNA insert has been identified which presumably contains the gene for the splicing endo- nuclease.

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1450 THE RAD1 GENE OF SACCHAROMYCES CEREVISIAE, Elizabeth Yang and Errol C. Friedberg, Stanford University, Stanford, CA 94305

The excision repair gene, RAD1, of Saccharomyces cerevisiae, has been cloned and sequenced. The coding region of the RAD1 gene is 2916 bp or 972 amino acids in size, which would translate into a protein with a theoretical molecular weight of 110kDa. The RAD1 transcript has been identified, and RNA hybridizations comparing the level of RAD1 mRNA to other known mRNAs show that in wildtype cells the expression of the RAD1 gene is very low. The 5' and 3' ends of the RAD1 mRNA have been mapped, and RNA protection experiments reveal one major transcript start in wildtype cells. Antibodies have been raised to two synthetic peptides, each representing a different region of the RAD1 amino acid sequence that is hydrophilic and has a high probability of containing a β turn. When used in Western blot analysis of total yeast lysates, both antisera identify a peptide specific band that migrates at 50kDa, rather than the predicted size of 110kDa.

The Nucleus and DNA Replication

1451 CONTROL OF 2 MICRON CIRCLE PLASMID PROPAGATION, James R. Broach[1], Ling-Chuan C. Wu[2] and Paul Fisher[2], [1]Department of Molecular Biology, Princeton University, Princeton, NJ 08544, and [2]Department of Pharmacology, Health Sciences Center, State University of New York, Stony Brook, NY 11794.

As an autonomously propagating genetic element, the multicopy yeast plasmid 2 micron circle can be viewed as a chromosomal replicon on which are superimposed plasmid encoded stability functions. Under steady state growth conditions the plasmid behaves essentially as a chromosomal replicon: each plasmid replicates once and only once during each cell cycle, using the same enzymatic machinery used to replicate chromosomal DNA. In spite of stringent control of its replication, though, the 2 micron circle is stable during mitotic growth and is transmitted efficiently to all haploid progeny following meiosis. Current evidence suggests that the relatively high stability of 2 micron circle and of hybrid plasmids derived from it can be attributed to a plasmid encoded partitioning system and to the ability of the plasmid to increase copy number when its copy number is low.

We have recently defined the 2 micron circle encoded components that are necessary for stable plasmid propagation. This stability system consists of two proteins, encoded in plasmid genes designated REP1 and REP2, and two cis-acting sites, the replication origin of the plasmid and a second site designated REP3. REP3 consists of five and one-half direct tandem repeats of a 62 bp sequence and is apparently the site at which the REP proteins act to promote stabilization.

To explore the mechanism by which the REP system stabilizes plasmid propagation, we have examined the properties of the REP1 protein and the associations of 2 micron circle with subcellular components. We find that the REP1 protein copurifies with the nuclear pellet and nuclear matrix fractions of yeast. In addition, the 2 micron circle appears to be attached to the nuclear pellet fraction through specific sites on its genome. The particular site on the plasmid genome through which this attachment occurs appears to vary as a function of the cell cycle. Solubilization of REP1 protein from a nuclear pellet fraction is coincident with release of 2 micron circle from the nuclear pellet. Finally, REP1 protein appears to exhibit DNA binding activity with specificity for specific regions of the 2 micron circle genome. These results suggest that REP1 protein acts as a bivalent connector, promoting association of 2 micron circle with the nuclear matrix, or with some component that copurifies with the matrix. These results will be discussed in terms of the probable role of the REP proteins in plasmid partitioning and copy control.

1452 DISSECTION OF THE YEAST DNA REPLICATION APPARATUS. Judith L. Campbell, L. M. Johnson, A.Y.S. Jong, F. Srienc, K. Sweder, Divisions of Biology and Chemistry, California Institute of Technology, Pasadena, CA 91125; M. P. Snyder, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

A purely biochemical approach to understanding the complex DNA replication apparatus of eukaryotic cells is unlikely to give much insight, since one can never be sure that the reactions characterized in vitro mimic reactions of physiological significance. Therefore, it has been necessary to take a combined genetic and biochemical approach to the study of DNA replication. In prokaryotic systems, collections of temperature sensitive DNA replication mutants have been used, for instance, in conjunction with in vitro replication extracts, to purify the proteins encoded by these replication genes and to determine their activities. As an example of using a genetic defect to identify a replication protein, we have shown that the product of the CDC8 gene encodes thymidylate kinase.[1,2,3] Recently, new technologies allow a different approach. Proteins known to be involved in procaryotic replication and therefore likely to be involved in yeast, can be purified from yeast by biochemical assay. The purified protein can in turn be used to provide an oligonucleotide probe or an antibody probe for isolating the gene. In the yeast system, the cloned gene can be altered by in vitro mutagenesis, reinserted into the chromosome and the phenotype of the resulting mutants can allow evaluation of the role of the protein either in replication or the related pathways of repair and recombination. We have begun to use this approach and have cloned the gene for yeast DNA polymerase I and shown that it is involved in yeast DNA replication. Secondly, we have purified several single-strand DNA binding proteins and have isolated the gene for one of these. Other proteins have been purified to homogeneity and will be exploited in the same way.

In addition to studies on the enzymatic apparatus of replication, we have studied DNA sequences involved. A flow cytometric assay of plasmid stability has been developed and used to define the functional structure of the ARS1 element.[4]

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1453 GENETIC ANALYSES OF snRNAS AND RNA PROCESSING IN YEAST, Christine Guthrie, Roy Parker, Bruce Patterson, Nora Riedel, Trey Simmons, Harold Swerdlow, and Joseph Tamm, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

We are exploiting the unique genetic capabilities of Saccharomyces cerevisiae to determine the functions of small nuclear RNAs (snRNAs). Yeast is especially suited for this investigation because, as we have recently demonstrated ([1] and unpubl.), the genes encoding at least 6 of these RNAs are present in single copy, in striking contrast to the situation in higher organisms. Our initial approach is to clone the genes encoding these RNAs (designated SNR3, SNR4, etc.), disrupt them by insertion of a yeast selectable marker, and then replace the resident chromosomal gene with its disrupted counterpart and observe the resulting phenotype.

Surprisingly, SNR3 appears to be dispensable [2]. To test the hypothesis that this is due to a related snRNA which performs the same function, we made a partial deletion of the gene encoding snR4, whose 2D electrophoretic behavior is similar to that of snR3. While the snr3-, snr4- double mutant is also viable, it uncovers a third apparently related species, snR5. Experiments to make the SNR5 deletion, and if necessary construct the triple mutant, are in progress.

Analyses of other snRNAs have provided more dramatic results. Cells deleted for SNR10 exhibit cold- and osmotic-sensitive growth. Preliminary results suggest that at least one biochemical defect in this strain involves rRNA synthesis and/or processing. Finally, deletions of either SNR7 or SNR8 produce inviable haploids. In the former case, we have shown that letnality can be complemented in trans by a small subclone encoding only the snRNA; this experiment is in progress for SNR8. Our highest priority is now the generation of conditional alleles.

In addition, with these cloned genes in hand, we can now use a variety of approaches to directly investigate (1) the interaction of snRNAs with other cellular components (minimally, the snRNP proteins), as well as (2) their involvement in mRNA splicing per se, by taking advantage of cis-acting splicing mutations we have generated in a complementary study [3].

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1454 STUDIES ON THE REPLICATION OF YEAST CHROMOSOME III, Carol S. Newlon*, R. P. Green, K. E. Kim, T. G. Palzkill*, S. Synn and S. T. Woody, Department of Biology, University of Iowa, Iowa City, IA 52242

Rare recombination events between the homologous HML and MAT loci on chromosome III result in a stable, circular derivative of the chromosome containing approximately 200 kilobases (kb) of DNA (1). We have prepared a library of BamHI fragments of this chromosome in the URA3 vector YIp5 and have constructed a restriction map of the entire ring chromosome. Analysis of each cloned fragment for autonomously replicating sequences (ARS's) has revealed the presence of at least eleven ARS elements on the ring chromosome. Each of these ARS's is contained within a fragment of 500 base pairs or less. The number of ARS's and their spacing (3 to 70kb) are broadly consistent with the expected distribution of replication origins (2).

DNA sequence analysis has revealed that at least three of these ARS's contain sequences related to the consensus sequence (A/TTTTATGTTTA/T) found in other ARS's (3, 4). To investigate the relative strength of these ARS's, we have made use of a colony color assay in which the copy number of a plasmid containing CEN4, an ARS, and the weak ochre suppressor, SUP11, can be determined from the color of the colonies produced by an ade2-ochre strain carrying the plasmid (P. Hieter, C. Mann, M. Snyder and R. Davis, per. comm.). The rates of both 1:0 and 2:0 segregations of the plasmid are directly reflected by the frequency of pink/red and white/red half sectored colonies. The relative efficiences of the chromosome III ARS vary by at least a factor of thirty.

To determine whether ARS's are essential for normal chromosome stability, we are systematically deleting ARS's from the wild type chromosome III (350kb) and two circular derivatives, the 200kb ring chromosome (1) and a 66kb derivative (5) which contains only two strong ARS's. Deletion of a single ARS from the 66kb derivative destabilizes it significantly while deletion of the same ARS from the normal chromosome has no detectable effect.

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1455 BIOCHEMISTRY AND GENETICS OF UBIQUITIN, Daniel Finley, Engin Ozkaynak, Aaron Ciechanover and Alexander Varshavsky, M. I. T., Cambridge, MA 02139

Ubiquitin, a 76 residue protein, occurs in eukaryotic cells either free or covalently joined via its carboxyl terminus to a variety of protein species. Previous work suggested that ubiquitin may function as a signal for attack by proteinases specific for ubiquitin-protein conjugates[1]. We have found that the first enzyme of the ubiquitin-conjugation pathway is thermolabile in the cell line ts85, a mouse-derived cell cycle mutant[2]. Using ts85, we show that the degradation of the bulk of short-lived proteins in this higher eukaryotic cells is accomplished through a ubiquitin-dependent pathway[3]. The S. cerevisiae ubiquitin gene has been isolated and found to contain six consecutive ubiquitin-coding repeats in a heat-to-tail arrangement[4]. This striking sequence organization suggests that yeast ubiquitin is generated by processing of a precursor protein in which tandem repeats of the ubiquitin amino acid sequence are joined directly via the Gly-Met bond between the last and the first residues of mature ubiquitin, respectively. We will describe ubiquitin gene disruption experiments, and in addition, replacement of the natural, internally repetitive ubiquitin gene with an artificial, single-copy derivative. The proposed role of amino-terminal ubiquitination in selective protein degradation[5] is being tested using ubiquitin-lacZ gene fusions.

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4. Ozkaynak, E., Finley, D. and Varshavsky, A. Nature, in press.
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1456 CONTROL MECHANISMS OF CHROMOSOME MOVEMENTS IN MITOSIS.
Mitsuhiro Yanagida, Yasushi Hiraoka, Tadashi Uemura and Tatsuya Hirano, Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

Cold-sensitive mutations in α- and β-tubulin genes of the fission yeast S. pombe blocked the spindle formation at restrictive temperature [1-4] and the cells were arrested at later stages of mitosis. Condensed individual chromosomes were seen in the arrested cells [4-5], indicating that chromosomes could condense and individually segregate in the absence of spindle function. In a highly reversible B-tubulin mutant incubated at restrictive temperature and then shifted to permissive temperature, spindle was rapidly formed and the chromosomes were completely separated within 20 min after shift [4].

A contrasting phenomenon was found in the case of type II DNA topoisomerase (top2) mutants that also blocked the nuclear division [6]. Neither chromosome condensation nor segregation occurred in the mutant cells at restrictive temperature. Transient double-strand cutting appeared to be required for the condensation-segregation processes. Spindle was, however, formed in top2 apparently at a normal timing, pulling the small parts of chromosomes but failed to separate them. Then spindle disappeared and the septum was formed, cutting across the undivided nucleus and producing two separated parts of the cell. The results clearly showed that top2 mutations block the chromosomal condensation-segregation but do not interfere the spindle formation and cytokinesis. At least parts of the pathways for chromosome condensation-segregation and spindle formation-dynamics appear to be independent. Type II enzyme appears to play a dual role in the nuclear division and also in the maintenance of a proper superhelical density in chromatin that was shown by its ability to compliment defect in type I enzyme [6].

By a cytological screening method, we have isolated new temperature-sensitive mutants that are not normal in chromosome movements in mitosis. Some mutants showed defective phenotypes nearly identical to those of tubulin and top2 mutants, but were derived from different genes. Investigation of these gene products and functions are being conducted.

[1] Toda et al. (1983) J. Mol. Biol. 168, 251-270. [2] Umesono et al. (1983) J. Mol. Biol. 168, 271-284. [3] Toda et al. Cell 37, 233-242. [4] Hiraoka et al. (1984) Cell 39 in press. [5] Umesono et al. (1983) Current Genetics 7, 123-128. [6] Uemura et al. (1984) EMBO Journal 3, 1737-1744.

Chromosome Structure

1457 MUTATIONAL ANALYSIS OF SPINDLE POLE BODY REGULATION, Peter Baum, Loretta Goetsch, and Breck Byers, Department of Genetics, University of Washington, Seattle, WA 98195

The microtubule-organizing organelle of yeast -- the spindle pole body (SPB) -- plays essential roles in various processes throughout the life cycle. Both karyogamy and spore wall formation, for example, are mediated by distinct functional states of the SPB. In the cell division cycle, the phenotypes of the cdc mutations reveal that SPB behavior is interdependent with other landmark events, reflecting the crucial role of the SPB in spindle formation. Despite continued RNA and protein synthesis and cellular enlargement, failure of nuclear division in cdc mutants is accompanied by the absence of further SPB duplication.

We have recently sought new ts variants which affect SPB behavior and have identified the mutation espl (for extra spindle pole bodies) that causes multiple rounds of SPB duplication within individual nuclei. This deregulation of SPB duplication with respect to nuclear division has been explored by assaying the phenotypes of various $espl^{ts}$ $cdcx^{ts}$ double mutants. We find that espl is epistatic, with regard to extra rounds of SPB duplication, to cdc4, cdc7, and cdc35, suggesting that the absence of continued SPB duplication by these cdc mutants results from an inhibitory mechanism mediated by $ESP1^{+}$. In contrast, the espl cdc28 double mutant is arrested with a single SPB; similarly, temperature-sensitivity for espl fails to permit continued SPB duplication in MATa cells subjected to arrest by mating factor. Therefore, a mechanism independent of ESP1 must be required for inhibition of SPB duplication at "start". Cloning of the genes involved in SPB duplication may permit us to determine their mode of regulation.

1458 CENTROMERE STRUCTURE AND FUNCTION, John Carbon, Louise Clarke, Hanspeter Amstutz, Barbara Fishel, Bruce Futcher, and Ray Ng, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

Centromere DNA's (CEN) have been isolated from the budding yeast, Saccharomyces cerevisiae, and the fission yeast, Schizosaccharomyces pombe. Nucleotide sequence comparison (1) of several S. cerevisiae CEN regions reveals the presence in each of a high (93-94%) A+T region of nearly uniform length (82-89 bp), designated sequence element II. This region is flanked on one side by a highly conserved 11 bp sequence (III), and on the other side by a less highly conserved 14 bp sequence (I). Hybridization with synthetic element III probes reveals the presence of similar DNA sequences in the centromere regions from S. pombe chromosomes I and II. In vitro mutagenesis studies on the S. cerevisiae CEN3 region have been carried out. Single base changes either within (2) or closely adjacent to the conserved sequence element III region can impair CEN function, resulting in increased frequency of mitotic nondisjunction of the altered chromsomes. Deletion experiments indicate that some DNA sequences to the right of element III are required for proper mitotic function, but the active CEN region is probably no longer than 150-200 bp. Both regions II and III are required for normal centromere function, although centromeres containing III plus truncated or rearranged portions of region II retain partial activity (3). These structural changes of the high A+T region II drastically affect the meiotic segregation patterns of the altered chromosomes; the sister chromatids separate prematurely in meiosis I and segregate randomly, giving some second-division segregation of CEN-linked markers. Thus, an intact element II region is necessary to hold the sister chromatids together during the first meiotic division.

Plasmids containing intact centromeres from either S. cerevisiae or S. pombe are present in low copy number (1 or 2 per cell) in the homologous host cell. Studies with S. cerevisiae containing as many as five selectable CEN plasmids per cell indicate that the extra centromeres interfere with nuclear division, cause increased chromosome nondisjunction, and lead to cell death, presumably due to competition for a limited number of binding sites on the mitotic spindle. Centromere plasmids in S. cerevisiae are mitotically stabilized and undergo proper Mendelian segregation in meiosis. However, S. pombe CEN plasmids replicating in S. pombe cells are not mitotically stabilized and do not survive through meiosis. This is consistent with the known intolerance of S. pombe cells to the presence of aneuploid chromosomes.

(1) For review, see Carbon, J., Cell 37, 351-353 (1984); (2) McGrew, J. and Fitzgerald-Hayes, M., Abstracts, XIIth Int. Conf. Yeast Genetics and Mol. Biol., p. 47 (1984); (3) Carbon, J. and Clarke, L., J. Cell Sci. Suppl. 1, in press.

1459 MITOTIC CHROMOSOME TRANSMISSION, Douglas Koshland, Michael Carson and Leland Hartwell, University of Washington, Seattle, WA 98195

The replication and segregation of chromosomes during mitotic cell growth depends upon the interaction of the chromosomes with specific proteins. We are developing novel assays for identifying genes that encode these proteins, the site(s) of interaction between these proteins and the DNA and the function of these interactions in chromosome replication and segregation. Many of the genes identified by cell division cycle mutations may encode proteins that interact with the chromosomes; therefore we have begun the analysis of these genes with our assays. A premise to our first assay is that many of the interactions between chromosomes and proteins will involve the metabolism of the DNA (introduction and removal of nicks, gaps, double strand breaks, modifications, etc.) and that the failure to complete any of these metabolic steps in its normal time frame may result in damage to the chromosomes. We may detect this damage because its repair by a recombination pathway will induce mitotic recombination while the absence of its repair may induce chromosome loss. Therefore we expect many CDC mutations that limit the function of proteins that metabolize DNA to induce chromosome recombination and/or loss. Furthermore, the map position of the mitotic recombinants induced by a particular CDC may indicate the distribution of the damage on the chromosome and thus the site(s) of chromosomal metabolism. We find that limiting the function of any CDC gene product in the nuclear pathway with the exception of start mutations induce chromosome recombination, loss, or both. Limiting CDC15 and CDC17 function induces recombination preferentially near the centromere and telomere respectively, suggesting that important metabolism of these two chromosomal domains fails to occur at these blocks. Subsequent biochemical analysis of telomere structure in CDC17 shows that telomere metabolism is altered in cells limited for CDC17 function. We are also using a second assay that may allow us to determine if the protein DNA interaction functions in replication or segregation. This assay can detect the simple loss, nondisjunction, over-replication and rearrangement of minichromosomes that may result from limiting cdc function. So far our results show that the minichromosome frequently over-replicates when limited for CDC6 function suggesting that this gene product is necessary for controlling the initiation of new rounds of DNA synthesis.

1460 MECHANISMS THAT ALTER CHROMOSOME STRUCTURE, Thomas D. Petes, Mark Goeble, Sue Jinks-Robertson and Arthur Lustig. Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637

Although the eukaryotic chromosome is usually regarded as a stable structure, in the yeast Saccharomyces cerevisiae, a number of cellular mechanisms exist that can alter DNA sequences within the chromosome. One type of mechanism involved in changing DNA sequences is recombination between repeated genes on nonhomologous chromosomes. I will discuss the evidence that recombination between yeast genes repeated on nonhomologous chromosomes occurs frequently in meiosis. A second mechanism that affects chromosomal sequences acts by altering the length of a simple sequence of DNA located at the end of the yeast chromosome. I will describe attempts to obtain strains of yeast that are mutant for this function. The third issue that will be discussed is the fraction of the yeast genome that is required for cell viability. We have investigated this problem by determining what fraction of random single gene disruptions in diploid yeast strains result in haploid lethality.

1461 TELOMERES AND ARTIFICIAL CHROMOSOMES OF YEAST, Jack W. Szostak, Toby Claus, Andrew Murray, Barbara Dunn, Dean Dawson and Victoria Lundblad. Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA. 02114

Inverted repeats composed of telomeric sequences can be processed into two new ends in yeast (telomere resolution). These new ends are subsequently modified by the addition of yeast-telomere sequences (the addition reaction). We have examined the substrate specificities of these two reactions. Inverted repeats composed of clusters of C4A2 repeats (Tetrahymena telomeres) or CA repeats are resolved. As few as 6 C4A2 repeat units are sufficient. The reaction is orientation specific, in that the center of the inverted repeat must correspond to the 5' end of the CA strand. We constructed inverted repeats with varying amounts of asymmetric DNA inserted at the center of symmetry, to prevent the formation of cruciforms. These structures also resolved, although large inserts decrease the efficiency of the reaction. It therefore seems likely that the resolution reaction involves a DNA cutting enzyme that recognizes CA rich DNA sequences.

Ends composed of telomeric sequences generated in vivo by the resolution of an inverted repeat, or in vitro by restriction digestion, are processed in vivo by the addition of several hundred base pairs of C1-3A DNA in an apparently non-template directed form of DNA synthesis. The reaction is independent of the recombination and repair gene RAD52. The added DNA is sometimes separated from the C4A2 cluster by linker sequences used in the plasmid construction, suggesting that strand invasion followed by primer extension is not the

mechanism of telomere elongation. A possible mechanism for this reaction, and a model of its role in telomere replication will be presented.

Artificial chromosomes can be constructed by combining cloned centromeres, replicators, telomeres and genes. Several experiments suggest that the size of these molecules is the critical factor in determining their mitotic stability. Increasing the size of an artificial chromosome up to >100 kb results in increased stability. A 130 kb fragment of a natural chromosome has a similar stability, about 100 fold less than the stability of the intact chromosome. Decreasing distance between the centromere and the closest telomere leads to only a small decrease in chromosomal stability. We conclude that the length of the DNA molecule is the main determinant of stability rather than the precise arrangement of functional elements.

Nucleus, Chromosomes and DNA Replication

1462 CHARACTERIZATION OF PLASMID pGCR1. Henry V. Baker II and Dan G. Fraenkel, Harvard Medical School, Boston, MA 02115

The levels of most glycolytic enzymes are approximately 5% of wild type in gcr strains grown on gluconeogenic carbon sources. Furthermore unlike wild type strains, there is an apparent inducibility of the affected enzymes when gcr strains are grown on media with sugars. Under these conditions the affected enzyme levels are approximately 20 to 50% of wild type. Kawasaki and Fraenkel reported the isolation of plasmid pGCR1 which encodes GCR complementing activity. Restriction endonuclease analysis revealed that the GCR complementing activity was carried on a 7.4 Kb fragment inserted at the BamHI site of plasmid pCV13. Sub-cloning experiments narrowed down the region encoding the GCR complementing activity to a 3.5 Kb fragment, and suggested that an NcoI site within this fragment was in the putative GCR gene. This suggestion was confirmed when a 3 Kb DNA fragment encoding LEU2 was inserted into the NcoI site. Furthermore, when chromosomal GCR was replaced with gcr::LEU2 the resultant strain showed the reported gcr phenotype. This result suggests that plasmid pGCR1 does in fact encode GCR. Work is now underway to determine the DNA sequence of GCR.

1463 CHARACTERIZATION OF CIS AND TRANS ELEMENTS ASSOCIATED WITH YEAST MT-DNA REPLICATION, Hugues Blanc, CGM du CNRS, Gif sur Yvette 91190, France.

In S. cerevisiae, a specific class of cytoplasmic mutants, the hypersuppressive rho^- (HS rho^-) which replicated preferentially their mt-DNA when in competition, enabled us to isolate three specific mt-DNA sequences of the rho^+ genome(wild type). The amplified mt genomes of the different HS rho^- carrying one of the three sequences, rep1, rep2 or rep3 300bp long and 85% homologous, contain the information recognizable by the enzymatic machinery to initiate DNA replication. The three functional rep sequences show several striking conserved features: (1) a nonanucleotide identical to the mt-DNA gene-promotor-like sequence, (2) immediately downstream a stretch of non alternated G (or C on the other strand), (3) and at the other extremity of the 300bp, a palindromic sequence of 40 nucleotides. The roles of these different modules in the initiation of DNA replication are under investigation. No mt-DNA encoded gene product seems to be absolutely required for replication, since every rho^- mutant can apparently replicate its mt-DNA accurately even though some of them have no DNA in common. Therefore all proteins critical for this process are probably nuclearly encoded. The synthesis of mt-DNA is regulated independently of the other yeast DNA replicons; consequently specific genes should be implicated in this phenomenon. So we set up a procedure to select t^s nuclear mutants which at elevated temperature are defective in mt-DNA propagation. Mutants of this type have been obtained and their analysis are in progress.

1464 EFFECTS OF POLYMYXIN B AND POLYMYXIN B NONAPEPTIDE ON YEAST. George Boguslawski, Biosynthesis Research Laboratory, Biotechnology Group, Miles Laboratories, Inc., Elkhart, IN 46515

Polymyxin B, a toxic, membrane-affecting antibiotic, can be rendered harmless to yeast cells by enzymatic removal of its fatty acyl moiety. The remaining cyclic peptide portion, polymyxin B nonapeptide, while not inhibitory to yeast, enhances sensitivity of cells to various drugs, presumably by increasing membrane permeability. Mutants resistant to polymyxin B simultaneously show decreased responsiveness to treatment with the nonapeptide. This indicates that the peptide portion of polymyxin B is the moiety responsible for the permeability changes. The resistance is inherited as a simple recessive trait.

Polymyxin B and PBN may become useful in yeast research for two reasons. First, there is a need for drug resistance markers in yeast to allow simple and rapid selection on complex media. Second, the PBN-induced perturbation of membrane function without damage to the cells may result in the increased efficiency of secretion of various molecules, and thus facilitate the recovery of desired products of cloned genes.

1465 THE USE OF YEAST TO SELECT PROBES FOR THE ANALYSIS OF A HIGHER PLANT GENOME. B.A. Bowen[1], J.H.Cramer[2], G.P.Creissen[1], S.R.Turner[1], H.Paaren[2], R.Brookland[2], G.E.Marks[1] and C.A.Cullis[1]. 1)John Innes Institute, Colney Lane, Norwich NR47UH,U.K. 2)Agrigenetics Corperation, Madison, Wisconsin 53716,USA.

The functional determinants of yeast chromosomal architecture can be defined as ars, CEN and telomeric sequences. We have selected fragments of DNA from the higher plant Luzula pilosa which mimic the phenotype of these sequences in yeast. This has proved to be an effective way of isolating fragments which can be used to probe the genomic organisation of this species. For example, a 3.6kb EcoRI fragment which contains two yeast ars sequences is derived from L.pilosa chloroplast DNA. As it hybridises strongly to at least one other L.pilosa fragment and to two non-contiguous regions of the chloroplast genome in both barley and maize which do not cross hybridise with each other, we think that it may contain a duplicated sequence, each copy of which flanks a segment of Luzula chloroplast DNA which is rearranged with respect to the genomes present in other monocots. CEN sequences confer mitotic stability on ars containing plasmids and reduce their copy number to one per cell. The yeast actin gene is apparently lethal at high copy number, so we have used a pJDB219::yeast actin vector (pBA6) to select sequences from both yeast and L.pilosa DNA which reduce plasmid copy number. The vector alone does not transform yeast at a measurable frequency($<10^{-10}$), but transforms at a high frequency when ligated to a fragment containing CEN4. We also have transformants containing L.pilosa DNA ligated to BclIcut pSZ216 (a linear vector bordered by two Tetrahymena telomeres). The cut vector lacks 1 telomere so does not transform yeast.

1466 'N ELECTROPHORETIC KARYOTYPE OF YEAST, Georges F. Carle, Maynard V. Olson, Department of Genetics, Washington University, St. Louis, MO 63110

A new method of separation for high molecular DNA molecules has been used to establish an "electrophoretic karyotype" of Saccharomyces cerevisiae. The separation and characterization of individual yeast chromosomes was performed by Orthogonal-Field-Alternation Gel Electrophoresis followed by transfer to nitrocellulose membrane and hybridization to single-copy yeast DNA probes. In our standard laboratory strain, twelve individual bands can be detected by ethidium-bromide staining of the gels; nine of these bands appear to arise from a single chromosome, the other three being multiplets. Interstrain chromosome-length polymorphism has been used to resolve two of the doublets into singlets.

1467 EVOLUTION OF THE YEAST SUC GENE FAMILY BY REARRANGEMENTS OF CHROMOSOME TERMINI, John Celenza, Frank Eng, Laura Sarokin, and Marian Carlson, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

The yeast genome contains a dispersed family of structural genes for invertase, the SUC genes (SUC1-SUC5 and SUC7). Most yeast strains carry zero, one or a few SUC^+ genes and carry negative alleles, called suc^0, at the remaining loci. The suc^0 alleles contain no SUC gene sequences, except for $suc2^0$, which is a pseudogene. Analysis of the physical structures of different SUC loci has shown that, except for SUC2, the SUC genes are located near telomeres and suggests that the evolution of this gene family occurred by at least two different mechanisms. Sequence comparison indicates that the homology between SUC2 and the other SUC loci includes only the structural gene, close flanking sequences and a 5' regulatory region distant from the gene. In contrast, the SUC1, SUC3, SUC4, SUC5, and SUC7 genes are embedded within large regions of homology. The homology extends several kb upstream from the SUC structural genes and extends downstream into telomere-adjacent sequences located approximately 5 kb from each SUC gene. Recovery of cloned DNA has shown that when a given chromosome carries a suc^0 allele rather than the corresponding SUC^+ allele, the chromosome has a different terminus lacking the SUC gene and flanking sequences. The evidence suggests that the dispersion of the SUC1, SUC3, SUC4, SUC5, and SUC7 genes to different chromosomes occurred by rearrangements of chromosome termini.

1468 CHARACTERIZATION OF CENTROMERE DNAs FROM SCHIZOSACCHAROMYCES POMBE, Louise Clarke, Barbara R. Fishel, Hanspeter Amstutz, and John Carbon, University of California, Santa Barbara, CA 93106

S. pombe centromere regions have been isolated by chromosome "walking" from the centromere-linked genes LYS1 on chromosome I and TPS13 and RAN1 on chromosome II. The putative centromere DNA segment from either chromosome, when present on an autonomously replicating plasmid, limits the high copy number of the plasmid to approximately one per cell. Both centromere DNA sequences hybridize to synthetic DNA probes derived from the highly conserved sequence element III region of Saccharomyces cerevisiae centromeres. The active segment from chromsome II is no longer than 2.5 kb. Integration of a plasmid carrying this DNA at the homologous site, duplicating the region in one copy of chromosome II in an h^-/h^- diploid, results in typical dicentric behavior. The host diploid undergoes an increased frequency of haploidization during mitosis, presumably initiated by chromosome non-disjunction. Unlike CEN sequences cloned on plasmids in S. cerevisiae, the S. pombe CEN sequences do not stabilize plasmids in mitotically dividing S. pombe cells, nor do they direct the proper segregation of plasmids through meiosis. This observation is consistent with the known instability of aneuploid chromosomes in this organism. Comparative DNA sequence data will be presented.

1469 STRUCTURE AND FUNCTION OF YEAST CENTROMERE III, Susan Cumberledge, Ray Ng, and John Carbon, Dept. of Biol. Sci., Univ. of Calif., Santa Barbara, CA 93106.

Experiments to define regions important for centromere activity as well as to determine the functional boundaries of CEN3 have been carried out. Point mutations in the conserved sequence element III region of CEN3 have been generated. Their affect on plasmid mitotic and meiotic segregation will be reported. In addition, we have generated HpaI and BglII cleavage sites 4 bp and 10 bp, respectively, to the right of element III and have used these to define the right-hand boundary of CEN3.

The meiotic behavior of ARS-CEN3 plasmids containing various centromere deletions(1) was assayed by tetrad analysis. Plasmids containing wild-type centromeres undergo proper $2^+:2^-$ segregation in at least 60% of the tetrads, always in the two sister spores. Tetrads showing $1^+:3^-$ or $3^+:1^-$ segregation are seldom observed (<8%). Plasmids containing 50 bp deletions in the high A+T element II show aberrant meiotic behavior. In 35% of the $2^+:2^-$ tetrads, the plasmid is found in non-sister spores. A $1^+:3^-$ or $3^+:1^-$ segregation is rarely observed. As the size of the deletion in element II decreases, the frequency of $2^+:2^-$ non-sister tetrads decreases. The presence or absence of element I has little effect on meiotic behavior. The above results suggest that element II is important for holding sister chromatids together during the first meiotic division.

(1) Carbon, J. and Clarke, L., J. Cell Sci. Suppl. 1, in press.

1470 SEQUENCES THAT PROMOTE AUTONOMOUS REPLICATION IN YEAST (ARS's) HAVE A CHARACTERISTIC ASYMMETRIC STRUCTURE, Andrew I. Dayton, G. Laws & C. Croce, The Wistar Institute, 36'th & Spruce St. Phila. Pa. 19104. ARS's are probably yeast DNA replication origins. To determine the sequences critical for function we selected for deletion mutagenesis a 236 bp human sequence with ARS acitivity in yeast, assaying for function by measuring the growth rates of transformants. The results indicated a pronounced asymmetry in ARS structure: Progressive deletion from one side resulted in a gradual progressive loss of function over 20 bp and defined the leftward border of a central "core" region essential for function. Progressive deletion from the other side resulted in a sharp progressive loss of function over a range of 3-4 bp, and defined the rightward border of the "core". The best three-way alignment between our 236 bp human ARS sequence and two short sequences, HO ARS and f82 (Kearsey 1983, *EMBO J* 2,p1571) precisely aligned our "core" with one similarly determined by Kearsey (1984, *Cell* 37,p299), whose data reflects the same asymmetry we measure quantitatively. Our results confirm that the Broach consensus sequence (J.R.Broach et al, 1980, *Cold Spring Harbor Symp. Quant. Biol.* 47,p1165) is not required for ARS function, place constraints upon what sequences may be considered necessary for ARS function and suggest a model in which the yeast replication machinery initiates DNA replication by first binding to a core region and then melting the DNA to one side of the core, initiating synthesis in the direction of the melt. Replication would initiate in the same direction as HO transcription.

1471 TOXIC EFFECTS OF EXCESS CLONED CENTROMERES, Bruce Futcher and John Carbon, University of California, Santa Barbara.

Plasmids carrying a yeast centromere have a copy number of one, while other yeast plasmids have high copy numbers. To find the reason for the low copy number of cen plasmids, several plasmids each carrying a centromere and a different selectable marker were constructed. When yeast were transformed with five different plasmids simultaneously, several effects on the host cells were noted. First, the plasmids were mutually unstable. Second, the host cells grew more slowly than control cells, even in non-selective medium. Third, cultures contained many dead cells. Fourth, the rate of chromosome loss increased two to six fold. Fifth, an unusually large proportion of the cells were in, or were about to begin, nuclear division. Sixth, there are preliminary indications that the copy number of each cen plasmid goes up in a strain containing 5 cen plasmids. Seventh, meiotic segregation was somewhat aberrant, and some spore clones contained cen plasmids which acted like ars plasmids in the first few mitotic divisions. Based mainly on the slow growth rate of the cells, we conclude that an excess of centromeres is toxic, and that the copy number of centromere plasmids is one because of selection against cells carrying multiple centromere plasmids. This toxicity may be due to competition between the centromeres for some factor present in limiting quantities; e.g., centromere binding proteins, microtubules, or space on the spindle pole body.

1472 THE FRACTION OF THE YEAST GENOME ESSENTIAL FOR CELL VIABILITY, Mark Goebl and Thomas Petes, University of Chicago, Chicago, IL 60637

We are determining what fraction of the yeast genome is essential for viability. To study this problem, we have constructed random gene disruptions in a diploid yeast strain and are determining the fraction of these disruptions which are haploid lethal. If the tetrads of the disrupted yeast segregate 2dead:2live spores, we assume that the disruption is lethal in haploids. In those disruptions that are not haploid lethal, we have checked respiratory competence and auxotrophy. We have examined 10 random disruptions thus far, and only one of these disruptions was a lethal event suggesting that a large fraction of the yeast genome is not essential for growth. This is a surprising result because 90% of the yeast genome is single copy DNA and most of the genome is transcribed. Although only one of the disruptions is lethal, 50% of the disrupted yeasts have altered phenotypes(such as a reduced ability to germinate or an inability to grow on glycerol). We conclude that a large fraction of the yeast genome is involved in important cellular functions although the sequences are not essential for growth.

1473 A GENETIC ANALYSIS OF HISTONE FUNCTION IN YEAST, Michael Grunstein, Tillman Schuster and Joonho Choe, Department of Biology and Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Changes in chromosome structure are central to the control of gene activity in eukaryotes. How these changes are caused is unknown and is the focus of our laboratory. Since histones form the building blocks of the chromosome we are pursuing a genetic and biochemical approach toward histone function in yeast. Experiments will be presented which define the regions in histones H2A and H2B which are required for chromosome function. Similar experiments are currently underway using histone H4. Our main conclusion to date is that the only essential portion of histone H2B (or H2A) is the central hydrophobic core of the protein. Other regions, including both evolutionarily divergent and invariant residues, are not required for cell viability.

Since S. pombe is a yeast whose chromosome undergo more obvious condensation than those of S. cerevisiae, we have characterized both structure and genetic properties of histones in this organism. Experiments will be presented that show the structure of S. pombe histones and the ability of these proteins to function in vivo in S. cerevisiae.

1474 STRUCTURAL REQUIREMENTS FOR A YEAST CENTROMERE, Alison Hill and Kerry Bloom, University of North Carolina, Chapel Hill, NC 27514

The centromeres of yeast chromosomes are characterized by short elements of DNA sequence homology that are organized in a 220-250 bp protected core in chromatin. This unique chromatin conformation is also associated with the elements of centromere homology in chromosome 4. We have isolated a 650 bp DNA fragment that spans the protected centromere core from chromosome IV and substituted these sequences into the centromere region of chromosome III. The CEN4 fragment is functional in either orientation in chromosome III and is organized in the same chromatin structure in chromosome III or IV.

In an effort to study the relationship of this unique chromatin structure to centromere function, we have constructed an inducible centromere by placing CEN3 sequences adjacent to the yeast GAL1 promoter. Growth on galactose induces transcription from the promoter,and inactivates CEN function. Conversely, growth on glucose represses transcription and the CEN3 sequences behave as stabilizing elements. The structure of the CEN3 region in chromatin is correspondingly destroyed upon transcriptional inactivation. We are examining the utility of these constructions to isolate conditionally high-copy centromere plasmids from yeast.

1475 DNA TOPOISOMERASE II IS REQUIRED AT THE TIME OF MITOSIS, Connie Holm and David Botstein, M.I.T., Cambridge, MA 02139

Although eukaryotic topoisomerases are well characterized in vitro, their function in vivo remains much less clear. To investigate this question, we produced five temperature-sensitive mutations of topoisomerase II by subjecting the cloned structural gene (Goto and Wang, Cell 36: 1073, 1984) to in vitro mutagenesis. These top2 mutants do not exhibit a uniform cell cycle arrest at restrictive temperature (see also DiNardo, Voelkel, and Sternglanz, PNAS 81: 2616, 1984), but 90% of the cells become inviable when an exponentially growing culture is held at restrictive temperature for 1 1/2 generations. This lethality is completely ameliorated when the cells are prevented from traversing the cell cycle by arrest with alpha factor. Thus, topoisomerase II is required in actively metabolizing cells only if they are passing through the cell cycle. When temperature-sensitive top2 cells are arrested with alpha factor and then allowed synchronously to traverse the cell cycle, they abruptly become inviable 60 to 80 minutes after release from alpha factor. Staining of the nuclei and microtubules shows that this is the time of mitosis, thus suggesting that topoisomerase II activity is required for chromosome segregation. Further experiments with the microtubule-destabilizing drug nocodazole show that when mitosis is prevented, inviability at the restrictive temperature is also prevented. These results are consistent with the hypothesis that topoisomerase II is required to allow the segregation of chromosomal DNA molecules. We are currently isolating suppressors of top2 mutations in order to identify cellular components that interact with topoisomerase II.

1476 ANALYSIS OF NDC1, A GENE REQUIRED FOR CHROMOSOME SEGREGATION, M. Andrew Hoyt, James H. Thomas and David Botstein, M.I.T., Cambridge, MA 02139

We have identified three cold-sensitive (cs) lethal alleles of a gene, (NDC1, for nuclear division cycle) which is required for the proper segregation of chromosomes at mitosis and meiosis. When an unbudded haploid ndc1cs cell is shifted to non-permissive temperature, our results indicate that the cell buds normally, replicates its DNA and duplicates and segregates its spindle pole body. However, nuclear DNA does not segregate, remaining in a single region in one cell body. The cell can then divide, producing one viable daughter cell that is diploid and one "aploid" daughter cell containing no detectable nuclear DNA.

We have isolated DNA containing NDC1 by complementation of the cs mutations. The gene is located on chromosome 13 within 800 bp of RAD52. Gene disruption experiments have demonstrated that NDC1 is essential for spore germination. While the cs alleles of NDC1 behaved as recessive to wt, diploids heterozygous for a disruption of NDC1 grew poorly. Faster growing "pseudo-revertants" harbored changes in ploidy which resulted in a higher number of chromosome 13 copies per genome. A hypothesis consistent with the known properties of ndc1cs cells is that their defect is in attachment of the chromosomes to one of the spindle poles. The defect could be in a component of the spindle pole body or kinetochores. For this reason, we are raising antibodies in order to localize the NDC1 product in the cell.

1477 TOPOISOMERASES I AND II AFFECT THE SUPERCOILING OF 2 μm PLASMID DNA IN VIVO, Joel A. Huberman and Raul A. Saavedra, Roswell Park Memorial Inst., Buffalo, NY 14263

The isolation and initial characterization of mutants in yeast DNA topoisomerases I and II by the Sternglanz laboratory [J. Biol. Chem. 259:1375-1377 (1984) and Proc. Natl. Acad. Sci. 81:2616-2620 (1984)] provides an important tool for investigation of the in vivo functions of these enzymes. We have used mutants provided to us by the Sternglanz laboratory to ask whether either topoisomerase I or II plays a role in maintaining supercoiling of topologically restrained DNA in vivo. We have isolated covalently closed circular 2μm plasmid DNA under conditions designed to prevent residual DNA topoisomerase action from wild type yeast cells and from yeast cells with topoisomerase I and/or II mutations (top1 and/or top2). The cells were maintained at 23° or incubated at 38° (non-permissive for top2 mutation) for 150 min prior to DNA isolation. Relative levels of supercoiling were determined by electrophoresis in agarose gels containing chloroquine phosphate. Several top1 alleles cause a slight (1-3 supercoils) increase in negative supercoiling while a top2 allele has no significant effect. However, preliminary results suggest that the presence of top1 and top2 mutations in the same cell leads to a much larger (>5 supercoils) increase in negative supercoiling at 38°. The results are consistent with the active supercoiling model recently proposed by the Worcel laboratory [Cell 37:21-41 (1984)] with the modification that topoisomerase II, like topoisomerase I, can relax supercoiled DNA in vivo.

1478 *ADDITIVE INTERACTIONS BETWEEN POLYMERIC MITOCHONDRIAL DNA-DERIVED ARS ELEMENTS AND CHROMOSOMAL CENTROMERES*, Bradley C. Hyman, Department of Biology, University of California, Riverside Ca. 92521

Individual eukaryotic cell chromosomes are organized into multiple replication units. To investigate the replicative behavior of molecules containing several replicons, hybrid plasmids were constructed that contain an increasing dosage of tandemly repeated ARS elements. The amplified nature of ARS$^+$ petite (ρ^-) mtDNA provides a ready source of tandemly repeated ARS segments. HS416 ρ^- mtDNA, which contains the repl sequence, was partially digested with TaqI to produce a population of fragments, each an integral multiple of the 700 base pair repeating unit. After fractionation on agarose gels, fragments of the desired ARS dosage were excised, ligated into the ClaI cloning site of YIp5, and introduced into ura3$^-$ yeast hosts. TaqI cleavage does not interrupt ARS activity, as YRMp416 DNA (YIp5 plus a single HS416 repeat) promoted high frequency yeast transformation to Ura$^+$ with subsequent unstable maintenance as an extrachromosomal element. While YRMp416 is maintained at high copy number within 5% of the cell population, mitotic stability is doubled to 10% when a HS416 dimer is present (YRMp416^2). The presence of a CEN3 sequence inserted into each of these plasmids does not efficiently stabilize these molecules. Rather, YRMp416-CEN3 is retained in 20% of a growing cell population, while YRMp416^2-CEN3 is present in over 40% of the cells, consistent with ARS dosage. Cellular copy number determinations will be reported to test the cis-dominant nature of centromere copy number control with these ARS elements. This approach will address the possibility of additive replication efficiency or enhanced segregation as a function of ARS dosage.

1479 ISOLATION OF DNA POLYMERASE I GENE. Lianna M. Johnson and Judith L. Campbell, Department of Chemistry, California Institute of Technology, Pasadena, CA 91125

Of the three DNA polymerase activities found in S. cerevisiae, DNA polymerase I is thought to be the most likely candidate for the nuclear replication enzyme. We have isolated the gene for DNA polymerase I by screening a yeast λgt11 library[1] with antibodies to polymerase I. The genomic clone was then isolated from a YEp24 library and shown to overproduce polymerase activity 4-5 fold. To examine whether DNA polymerase I is a single copy essential gene, a gene disruption experiment was performed in a diploid strain. Sporulation resulted in 2 viable and 2 inviable spores, demonstrating that DNA polymerase I is in fact a single copy essential gene. The spores containing the disrupted gene germinate to give a terminal phenotype typical of a replication defect--dumb-bell shaped cells.

1. M. P. Snyder, personal communication.

1480 CLONING OF THE DNA MODIFICATION METHYLASE Hha II IN SACCHAROMYCES CEREVISIAE, Brigitte L. Lebreton and Paul H. Roy, Laval University, Québec, Canada.

A hybrid plasmid has been constructed consisting of the yeast expression vector pAAH5, which promotes high levels of transcription from an ADC1 promoter adjacent to a Hind III site and a 851-bp Aha III fragment containing the Hha II modification methylase gene. The latter fragment was derived from pDI 10, in which the Hha II methylase gene is expressed from a distant pBR 322 promoter. The Hind III ends of the vector were completed with DNA polymerase I large fragment and the cloned fragment added by blunt-end ligation. Transformants of E. coli were selected by colony hybridisation using a probe prepared from an M13 clone of the 851-bp fragment. These clones do not express the methylase gene in E. coli, confirming the absence of a bacterial promoter and indicating that the ADC1 promoter may be non-functional in E. coli. DNA prepared from these clones was transformed into yeast. Transformation into S. cerevisiae LL20 (∝, leu 2-3, leu 2-112, his 3-11, his 3-15) was carried out using the LiAc-treated cells technique. DNA was prepared from transformants and analysed for the presence of the Hha II methylase gene and its expression. Partial or full protection of endogenous DNA to Hinf I (isochizomer of Hha II) digestion was found in some transformants, indicating the expression of the Hha methylase gene. Further investigation is needed to learn more about the level of expression in transformants.

This is the first example of the introduction of a foreign site specific DNA methylase activity in an eucaryotic cell which methylates the majority of the cell DNA under the control of a yeast promoter.

1481 GENES CONTROLLING TELOMERE LENGTH IN SACCHAROMYCES CEREVISIAE, Arthur J. Lustig and Thomas D. Petes, Dept. of Molecular Genetics & Cell Biology, The University of Chicago, Chicago, IL. 60637

DNA located at the telomeres of yeast chromosomes is present as $(C_{1-3}\ A)_n$ tracts from 300 to 700 bp in length. The size of these tracts varies to a small extent within a single telomere (±50 bp) and to a much larger degree (2-fold) between some yeast strains. Recent data from our laboratory (R. M. Walmsley and T. D. Petes, in press) have shown that telomere length is under genetic control.

The mechanism by which yeast maintains telomere length as well as the possible functions of telomeric sequences is being investigated through the identification and characterization of mutants defective in specific genes controlling telomere length. To screen for such mutants we have used a bank of yeast mutants temperature sensitive (ts) for growth at 37°C. DNA was isolated from ∿200 members of the ts bank after shifting to the restrictive temperature. The size of the telomeric $(C_{1-3}\ A)_n$ tract was ascertained directly by Southern blot analysis using a simple sequence probe. Five mutants demonstrating a dramatic increase or decrease in $(C_{1-3}\ A)_n$ tract length have been identified. The nature of these mutations in telomere structure is currently under investigation.

1482 LACK OF AN ASPERGILLUS PROMOTER FUNCTION AND INTRON SPLICING IN SACCHAROMYCES. Gary L. McKnight, ZymoGenetics, Inc., 2121 N. 35th Street, Seattle, WA 98103

The function in S. cerevisiae of an Aspergillus nidulans promoter and the proper splicing of an A. nidulans intervening sequence have been investigated. An A. nidulans cDNA and corresponding gene which encode alcohol dehydrogenase (ADH) have provided the test system. The A. nidulans ADH cDNA generates less ADH activity than the S. cerevisiae ADH1 cDNA when each is expressed from the S. cerevisiae ADH1 promoter. The first of two introns present in the A. nidulans ADH gene was tested for efficient splicing in S. cerevisiae following the replacement of the corresponding cDNA segment with the genomic DNA segment. The intron was not efficiently spliced, perhaps due to lack of a TACTAAC splicing signal. The A. nidulans ADH promoter did not efficiently express the A. nidulans ADH cDNA in glucose-grown S. cerevisiae. These results suggest the promoter and splicing signals in filamentous fungi are not similar to those employed in S. cerevisiae.

1483 INTERNUCLEOSOMAL CLEAVAGE OF YEAST CHROMATIN FROM CELLS TREATED WITH GLYCOPEPTIDIC BLEOMYCINS. C. W. Moore, C. Jones and L. Wall, University of Rochester School of Medicine, Rochester, NY 14642.

In our studies of the cellular and genetic control of bleomycin action on Saccharomyces cerevisiae and human diploid fibroblasts, we have investigated the action of bleomycin on yeast chromatin with the use of agarose gel electrophoresis. Diploid strain CM-1293 was grown to mid-logarithmic or stationary phase in complete nonsynthetic growth medium. Washed cells ($1-2x10^7$ per ml) were exposed to bleomycins at pH 4.5 in deionized water. No exogenous metal ions were added to the mixture. After treatments, cells were washed, converted to spheroplasts, and lyzed. DNA was extracted, purified and run on 1.5% horizontal or vertical agarose gels.

Electrophoretic analyses of DNA isolated from untreated cells exhibited a high molecular weight, indicating that endogenous nucleases did not significantly affect native yeast DNA. Whole yeast cells treated with bleomycin showed increasing DNA fragmentation and degradation with increasing concentration of bleomycin (10^{-7} to 10^{-3} M), indicating that DNA cleavage was dose-dependent; a series of DNA bands was also observed. The repeated unit length of these bands has been determined, and is consistent with preferential chromatin cleavage in yeast at internucleosomal or linker DNA.

1484 IDENTIFICATION AND CHARACTERIZATION OF S. cerevisiae CEN 14, Maureen Neitz and John Carbon, University of California, Santa Barbara, CA 93106

The nucleotide sequence of a small DNA fragment containing the functional centromere from S. cerevisiae chromosome XIV will be presented. Sequence data indicates that CEN 14 contains an (A+T)-rich region resembling the sequence, element II, observed in other sequenced yeast centromeres, as well as the highly conserved sequence, element III. Centromeric DNA was identified by its ability to stabilize a yeast plasmid against mitotic loss (segregation rate 0.02), and by the predominant 2+:2- segregation of such plasmids through meiosis. This DNA was localized to chromosome XIV by integrating a flanking DNA sequence along with a selectable gene and mapping the integrated gene by standard genetic methods.

1485 FURTHER CHARACTERIZATION OF SALT-TREATED RESIDUAL NUCLEI IN YEAST, Judith A. Potashkin and Joel A. Huberman, Roswell Park Memorial Institute, Buffalo, NY 14263

Isolation and initial characterization of salt-treated residual nuclei from Saccharomyces cerevisiae has previously been reported (Exp. Cell Res. 153:374). Here we report further characterization of these residual nuclei. The proteins associated with residual nuclei in yeast have been compared in cdc 7 and pep 4 mutants. The most abundant proteins are identical in both mutants and are of low molecular weight (60 Kd and less). There are no major protein bands in the molecular weight range of the lamin proteins. When yeast residual nuclei protein preparations are tested for immunological reactivity with antibodies which cross-react with lamins from several eukaryotes, there is no signal detected. In this respect, the polypeptide profile of yeast residual nuclei appears to be most similar to that of nuclear matrices in Tetrahymena. Electron microscopic thin sections of detergent-treated yeast nuclei, which are lacking nuclear membranes, show no evidence for a peripheral lamina.

The DNA and proteins associated with residual nuclei during different times in the cell cycle (G1, S and M) were also investigated. The major proteins associated with residual nuclei are the same at all time points tested though there were a few differences in less abundant proteins. Studies on the association of TRP1ARS1, CEN 6, 2 μ plasmid and rDNA with residual nuclei are in progress. Preliminary results suggest that these sequences associate with residual nuclei to different extents at different points in the cell cycle.

1486 CONDITIONAL YEAST VECTOR AMPLIFICATION, Rajinder S. Sidhu and Arthur P. Bollon, Wadley Institutes of Molecular Medicine, Dallas, Texas 75235

Yeast vector, pSB227, has 2μ ARS, and Trp1 and leu 2-d gene of yeast. Another isogenic plasmid, pSB228, has normal leu 2 gene in place of leu 2-d gene. In yeast cells grown in minimal media lacking tryptophan but supplemented with leucine, both plasmids were present at 20-30 copies per cell. When yeast cells were grown in minimal media lacking leucine, plasmid pSB227 increases to 200 copies per cell, while pSB228 was still present at 20-30 copies per cell. Since leu 2-d gene has most of the promoter deleted and is thus expressed very poorly in yeast, yeast cells compensate this defect by increasing the gene dosage albiet plasmid copy number. We have examined the expression of the normal leu 2 promoter and the leu 2-d promoter by constructing a leu 2 - lac z fusion and assaying B-galactosidase levels. Increased copy number per cell in media lacking leucine can either be due to selection of cells with high copy number or due to increased replication of plasmid. Experiments discriminating between these two possibilities will be presented.

1487 FRACTIONATION AND PARTIAL CHARACTERIZATION OF LARGE MOLECULAR COMPLEXES CONTAINING YEAST DNA REPLICATION ENZYMES, Rodger G. Smith, Michael D. Strathearn, John F. Scott, University of Illinois, Urbana, IL 61801.

We are using a multicopy yeast DNA plasmid YARp1 (Trp1 R1 Circle) as a model system for the study of the structure and function of Saccharomyces cerevisiae replicator chromatin. The plasmid is only 1,453 base pairs, present at more than 100 copies per cell, and is maintained in a chromatin structure comparable to yeast chromosomal DNA. In order to study protein complexes that may be associated with replicator chromatin, we are purifying YARp1 chromatin DNA from lysates made by osmotic shock of yeast spheroplasts at physiological ionic strength. The lysate is then subjected to several additional purification steps which include (in order): tangential flow ultrafiltration, ultracentrifugation, and gel filtration on Bio-Gel A-15M. Gel filtration yields a partially included peak of YARp1 chromatin of approximately 10 Md that is separated from the bulk of the protein. Additionally, several other enzyme activities including DNA and RNA polymerase, DNA primase, topoisomerase, and fumarase, were shown to elute as high molecular weight peaks. The DNA primase activity coelutes with the DNA polymerase activity at an apparent molecular weight of 1.8 Md, and may represent a larger molecular weight complex containing the two activities. This high molecular weight material has been pooled and further analysis has shown that the proposed complex elutes from Bio-Gel A-15M at a comparable position following either freeze-thawing or $(NH_4)_2SO_4$ precipitation at 50% saturation.

1488 YEAST HISTONE ISOLATION USING HPLC, Pamela C. Stacks and Joseph Mazrimas, San Jose State University, San Jose, Ca. 95192 and Lawrence Livermore National Laboratories, Livermore, Ca. 94550.

It is known that the core histones of yeast and higher eukaryotes exhibit different separation characteristics. We report here a comparison of yeast and calf thymus histone separations by reverse phase high performance liquid chromatography. Yeast nuclei were isolated, digested with micrococcal nuclease and pancreatic ribonuclease, lysed and crude nucleohistone complexes precipitated by addition of MgCl2. A histone rich fraction was prepared by acid extraction and ethanol precipitation. The histone fraction was easily suspended in aqueous 0.1% trifluoroacetic acid (TFA) and injected into a 70 cm PRP-1 reverse phase HPLC column (Hamilton).

All five calf histones were cleanly separated from each other upon elution with a gradient of 23 to 65% acetonitrile in 0.1% TFA. By monitoring the UV absorbance at 214nm, the order of elution was determined to be H1, H2b, H2a, H4, H3 with H3 having a retention time of approximately 40 minutes. Significant differences were observed in the calf thymus and yeast histone elution profiles. Yeast histones H2a, H4, and H2b all eluted earlier than calf thymus H2b. Yeast histone H3 was most strongly retained by the column. The identity of isolated fractions was confirmed by electrophoresis using standards.

1489 ROLES OF DNA TOPOISOMERASES I AND II IN DNA REPLICATION AND TRANSCRIPTION, Rolf Sternglanz, Stephen DiNardo, Steven J. Brill and Karen Voelkel-Meiman, SUNY, Stony Brook, NY 11794

Yeast strains with mutations in the genes for DNA topoisomerases I and II have been identified by us previously. Topoisomerase I mutants (top1) are viable and grow at almost a normal rate [JBC 259, 1375 (1984); also accompanying Abstract]. A conditional-lethal temperature-sensitive topoisomerase II mutant (top2-1) has been described [PNAS 81, 2616 (1984)]. The mutant is defective in the termination of DNA replication but otherwise appears to replicate and transcribe its DNA normally. In contrast to the single mutants, top1 top2 double mutants grow poorly at the permissive temperature and shut down DNA and bulk RNA synthesis rapidly at the restrictive temperature. Ribosomal RNA and tRNA synthesis are affected while the synthesis of 3 different mRNAs is unaffected. The results suggest that DNA replication and at least some RNA synthesis require topoisomerase action but that either topoisomerase can perform the required function (except for termination of DNA replication where topoisomerase II is essential for decatenation).

1490 CONSTRUCTION AND ANALYSIS OF DELETION CHROMOSOMES IN YEAST, Richard T. Surosky and Bik-Kwoon Tye, Cornell University, Ithaca, NY 14853

We have developed a simple method for the construction of large chromosomal deletions in yeast. Diploid yeast cells were transformed with DNA fragments that replace large regions of the chromosome by homologous recombination. Using this method, we have constructed a deletion on chromosome III in which the entire left arm has been removed. The centromere is 12 kb from the left telomere. We have also deleted the right arm of chromosome III producing a 120 kb chromosome. Both of these telocentric chromosomes have approximately the same mitotic stability as a normal chromosome III. We have also made systematic deletions on both the left and right arms of chromosome III. A 65 kb deletion chromosome shows a ten-fold decrease in mitotic stability. Smaller deletion chromosomes show further decreases in stability.

1491 DNA SEQUENCE-POSITIONED CHROMATIN STRUCTURES OF THE YEAST HSP83 LOCUS, C. Szent-Gyorgyi, D.B. Finkelstein, and W.T. Garrard, The University of Texas Health Science Center at Dallas, Dallas, Tx. 75235

We have investigated at high resolution the chromatin structure of the locus containing a Saccharomyces cerevisiae gene that encodes an 81 kd heat shock protein (the homolog of Drosophila HSP83). Northern hybridization enables us to determine that within 10 min of heat shock (39°) the steady state level of HSP83 transcript is induced at least tenfold over the basal level (27°). DNase I and micrococcal nuclease cutting sites in nuclei isolated from either heat shock induced or uninduced cells were mapped along the HSP83 locus by indirect end-labeling. A persistent set of nuclease hypersensitive features were noted. Regions ∿150 bp in breadth that are hypersensitive to DNase I are centered at -1625 bp, -545 bp, -155 bp, +2400 bp, and +3375 bp relative to the transcription start. The DNase I sites (except +2400 bp) each display centrally located protected regions of 20-40 bp, implying the presence of DNA binding proteins. The Pelham heat shock consensus sequence lies within a protected subdomain of the site at -155 bp. Sequences corresponding to poly(A) addition and possibly transcription termination reside within the site at +2400 bp. A clear array of DNA sequence-positioned nucleosomes, detectable by either nuclease, resides 3' to the DNase I hypersensitive region at +3375 bp; a similar array may exist 5' to the site at -1625 bp. These two hypersensitive regions are also each demarcated 5' and 3' by exceptionally strong micrococcal nuclease cleavages ∿ 160 bp apart, a length which coincides with the canonical nucleosomal repeat. (Supported by grants from NIH and The Robert A. Welch Foundation).

1492 **CHALLENGING NUCLEOSOME POSITIONS ON YEAST PLASMID CHROMATIN,** Fritz Thoma and Robert T. Simpson, National Institutes of Health, Bethesda, MD 20205

The TRP1/ARS1 circular plasmid has three precisely positioned nucleosomes on a sequence of unknown function (UNF) flanked by nuclease sensitive regions (Thoma et al. J.Mol.Biol. 177,715 [1984]). To test whether positioning is determined by boundary conditions or due to histone-DNA interactions we have inserted DNA fragments of varying lengths between the positions of two of the nucleosomes. In every case the nucleosomes originally resident on the yeast sequences were positioned on the same sequences. Insertion of 75 bp resulted in the additional sequences existing in a linker region; a 155 bp insert contained an additional nucleosome on the new DNA. When 300 bp was inserted, one additional nucleosome was present, flanked by linkers of 100 and 50 bp, even though the added DNA is long enough for two nucleosomes. Since long linkers are observed rather than altered nucleosome positions, we doubt that boundary conditions determine nucleosome position in this case. The 300 bp insert contains two tandemly repeated segments of DNA derived from a sea urchin 5S rRNA sequence which forms a phased nucleosome when assembled with histones in vitro (Simpson and Stafford, PNAS, 80, 51 [1983]). The nucleosome formed in yeast maps in a similar position on one of the tandem repeats; the same sequence in the other repeat is in a linker region. A nucleosome positioned on this second 5S sequence would impinge on one of the stable nucleosomes on the yeast UNF sequences and may therefore be precluded. We conclude that sequence specific DNA histone interactions may play an essential role in positioning of nucleosomes both in vivo and in vitro.

1493 CLONING, CHARACTERIZATION AND SEQUENCE OF THE YEAST DNA TOPOISOMERASE I GENE, Catherine Thrash[1], A.T. Bankier[2], B.G. Barrell[2], and Rolf Sternglanz[1], SUNY[1], Stony Brook, NY 11794 and MRC Laboratory of Molecular Biology[2], Cambridge CB2 2QH, England

The structural gene for yeast DNA topoisomerase I (TOPI) has been cloned from two yeast genomic plasmid banks. Integration of plasmids carrying the gene and subsequent genetic mapping shows that TOPI is identical to the gene previously called MAK1. Six topl (mak1) mutants including gene disruptions are viable, demonstrating that DNA topoisomerase I is not essential for viability in yeast. A 3787 bp DNA fragment including the gene has been sequenced. The protein has 769 amino acids and a molecular weight of 90,020.

1494 HIGH FREQUENCY EXCISION OF A TY ELEMENT DURING TRANSFORMATION OF YEAST, Gary Tschumper and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, California 93106

A characteristic feature of cloned yeast centromeres is their ability to restrain plasmid copy number in yeast to an average of one copy per cell. We have constructed a plasmid that permits selection of yeast transformants on the basis of plasmid copy number. In addition to the cloned centromere (CEN3), a selectable marker (LEU2) and 2 micron DNA, the plasmid contains the coding region of the kanamycin resistance gene from the bacterial transposon Tn5 attached to a fragment of Chlamydomonas reinhardtii DNA that functions as a weak promoter in yeast. In yeast, the phosphotransferase encoded by the kanamycin resistance gene confers resistance to Geneticin (G418 sulfate). Yeast cells containing this plasmid are sensitive to levels of Geneticin greater than 100 ug/ml, however, when the CEN3 copy number control is absent, the 2 micron DNA drives the plasmid to high copy number and the cells become resistant to more than 500 ug/ml of Geneticin. During initial experiments with this plasmid, a transformant was obtained in which resistance to high concentrations of Geneticin was conferred by a low copy number plasmid. Analysis of the plasmid revealed a Ty1 element inserted in the promoter of the phosphotransferase gene resulting in high level expression of this gene. When yeast is retransformed with this plasmid, about 25% of transformants carry a plasmid from which Ty1 has undergone specific excision, leaving behind a solo delta sequence and rendering the host cells sensitive to high levels of Geneticin. The frequency of Ty1 excision is greatly reduced following the initial transformation event.

1495 HIERACHY OF DNA REPLICATION ORIGINS IN YEAST. Bik-Kwoon Tye, Clarence Chan, Gregory Maine, Pratima Sinha and Steve Passmore. Cornell University, Ithaca, NY 14853.
We have previously reported the isolation of yeast mutants that are defective in the maintenance of circular minichromosomes. The minichromosomes are mitotically stable plasmids, each carrying an ARS and a centromeric sequence. We characterized 40 minichromosme maintenance defective (Mcm$^-$) mutants which comprise 16 complementation groups. These mutants can be divided into two classes, specific and nonspecific, by their differential ability to maintain minichromosomes with different ARSs. The specific class of mutants is defective only in the maintenance of minichromosomes that carry a particular group of ARSs. Two specific mutants, Mcm9 and Mcm46, have been analyzed further. Mcm46 shows temperature sensitivity for ARS specificity. At low temperature, it affects the stability of some but not all minichromosomes, depending on the ARS present. At high temperature, it affects all minichromosomes tested. The homozygous diploid of this mutant also loses its natural chromosomes at an abnormally high frequency. The mutant Mcm9, besides showing an Mcm$^-$ phenotype, is also an α specific sterile. These two phenotypes are caused by a single mutation mapped at chromosome XIII, 2cM from LYS7. The MCM9 gene has been cloned and its activity is localized within 400 bp of a DNA fragment. The functions of the products of the MCM9 and MCM46 genes will be discussed. To investigate the element responsible for the differential behavior of the different ARSs in the specific mutants, we determined the nucleotide sequence of 5 telomeric ARSs which are highly homologous and yet were affected differently in the specific mutants. We will report on the controlling element, which seems to be the site of interaction with the products of the MCM9 and MCM46 genes, within these ARSs.

1496 YEAST ACENTRIC RING PLASMIDS, Michael Woontner, Randy Strich, Mark Fagan, and John Scott, University of Illinois, Urbana, IL 61801
Virtually all yeast vectors in common use are shuttle plasmids harboring E. coli sequences. We have been using plasmids we call YARp's (for Yeast Acentric Ring plasmids) from which the foreign sequence has been eliminated. The YARp's thus far constructed are all derivatives of the TRP1 RI Circle [Zakian and Scott, Mol. Cell. Biol. 2:221-232, 1982], which we have renamed YARp1. We have made deletion and insertion derivatives in an attempt to delimit the sequence required for ARS1 function in the YARp context. Our smallest plasmid is only 880 bp and is not dramatically different, in terms of copy number and stability, from the parent 1453 bp plasmid. Although insertions which are not immediately adjacent to ARS1 are tolerated, insertions as small as 10 base pairs lower the stability and copy number if near the ARS. One large insertion is the 1.2 kbp HindIII fragment containing the URA3 gene. This insert interrupts the TRP1 sequence but still allows selection of transformants. Both the URA3 and TRP1 gene products are expressed from YAR plasmids at about 100 times the level found in wild-type cells. The copy numbers of various YARp's range from fifty to several hundred per haploid cell. In addition, these plasmids are extremely stable in comparison with other high-copy plasmids. The novel properties of these plasmids make them useful as cloning vectors as well as tools for studies of replication and gene expression. We are continuing our characterizations and are attempting to clone other genes and control sequences such as promoters and centromeres into the plasmids while retaining some or all of the useful YARp properties.

1497 IDENTIFICATION AND CHARACTERIZATION OF THE YEAST NUCLEAR MATRIX, Ling-Chuan C. Wu, James R. Broach[1], and Paul A. Fisher, Department of Pharmacological Sciences, S.U.N.Y. at Stony Brook, Stony Brook, NY 11794, and [1]Department of Molecular Biology, Princeton University, Princeton, NJ 08540.

Partially purified yeast (S. cerevisiae) nuclei have been subjected to exhaustive nuclease digestion followed by sequential extraction with 2 % Triton X-100 and 1 M NaCl. The 10,000 X g pellet remaining after these procedures is largely proteinaceous. SDS-PAGE analyses indicate that it is a biochemically distinct subcellular fraction, while EM examination demonstrates that it is composed of components that are morphologically similar to nuclear matrices similarly derived from a variety of higher eukaryotes. The polypeptide composition of the yeast nuclear matrix fraction is complex. Polypeptides range from 10-kD up to nearly 200-kD. Using an antiserum directed against yeast DNA topoisomerase II, we have been able to show that the topoisomerase polypeptide remains completely associated with the nuclear pellet fraction during both nuclease treatment and Triton X-100 extraction, but is largely soluble in 1 M NaCl. These analyses have been complicated by the fact that DNA topoisomerase II appears to be exquisitely sensitive to in vitro proteolysis during cell fractionation. Quantitative degradation from a polypeptide of 160-180-kD to a discrete form approximately 20-kD smaller has been observed in a number of preparations, and in the presence of various protease inhibitors. This proteolysis is of uncertain significance, both with respect to the association of DNA topoisomerase II with the yeast nuclear matrix, and to the structural integrity of the nuclear matrix, per se.

1498 SYNTHESIS, PROCESSING AND SECRETION OF FOREIGN PROTEINS IN YEAST, Geoffrey T. Yarranton, Clive R. Wood, Stephen Little, John Kenten, Michael Boss, and Spencer Emtage, Celltech Limited, 250 Bath Road, Slough SL1 4DY, U.K.

The yeast Saccharomyces cerevisiae is capable of synthesising and secreting higher eukaryotic proteins. Secretion may be directed either by a homologous or heterologous leader peptide, and secreted proteins are usually enzymatically active. Higher eukaryotic proteins synthesised but accumulated intracellularly in yeast are generally insoluble and inactive. The synthesis, processing, secretion and glycosylation of foreign proteins will be described, as well as the formation of functional antibodies in cells co-expressing both heavy and light chains.

1499 DOMINANT TRANSFORMATION AND PLASMID AMPLIFICATION IN SACCHAROMYCES CEREVISIAE

J.D. ZHU, R. CONTRERAS, W. FIERS, Laboratory of Molecular Biology, State University of Ghent, B-9000 Ghent, Belgium

The cDNA of the mouse dhfr was put under the transcriptional control of the cytochrome C or the actin promoters and terminators on 2μ-derived plasmids. It was possible to transform yeast cells with such plasmid by selection of MTX resistance and amplify the plasmid by applying higher concentration of MTX.

Secretion and Membrane Biology

1500 **MATURATION AND SECRETION OF THE M-dsRNA ENCODED YEAST PREPROTOXIN.** K.A. Bostian, Section of Biochemistry, Brown University, Providence, RI 02912.

The type I killer system in S. cerevisiae is characterized by secretion of a polypeptide toxin which kills sensitive cells by disrupting cytoplasmic membrane function. This toxin is encoded by the 1.9 kilobase pair double-stranded RNA killer plasmid, M_1-dsRNA, which also determines specific immunity to toxin.

M_1-dsRNA is found in cytoplasmic virus-like particles (VLP's) called ScV-M_1. A single capsid protein (88 kd) comprises more than 95% of the VLP protein. This capsid, called VL_{1A}-P1, is encoded by L_{1A}-dsRNA, a 4.5 kb dsRNA present in type 1 killers in separate VLP's (ScV-L_{1A}), also encapsidated by VL_{1A}-P1. ScV-M_1 is a satellite "virus" of ScV-L_{1A}, dependent on it for capsid production. No infectious cycle is demonstrable for these VLP's or dsRNA's, which are stably maintained at a copy number which is dependent on both the nuclear genetic background and on variation in the L_{1A}-dsRNA.

The secreted toxin has recently been shown to be composed of two dissimilar, 9.5 and 9.0 kilodalton (kd) disulfide-linked subunits, denoted α and β respectively (Bostian, et. al. 1984, Cell 36: 741). The protein dimer is synthesized as an intracellular 43 kd glycosylated precursor. The preprotoxin gene comprises four protein domains. In sequence they are δ, a 44 amino acid leader containing a typical secretion signal sequence (residues 6-27), α, an approximately 86 amino acid toxin component, γ, an approximately 103 amino acid component believed to determine immunity, and β, the C-terminal 83 amino acid toxin component. α and β are secreted as the disulfide-linked two component toxin. γ is glycosylated during protoxin maturation, and recent data confirms our prediction that glycosylation occurs at all three of its potential glycosylation sites. Its fate, and that of δ is unknown.

Expression of constructs involving M_1-cDNA and the PHO5 promoter demonstrates that δ plays no essential role in toxin production or immunity. During maturation of the normal dsRNA-encoded protoxin, δ removal does not occur prior to the Golgi phase of secretion. However, when the PHO5 signal peptide replaces most of δ to form a hybrid protoxin gene with M_1-cDNA, expressed under control of the PHO5 promoter, this signal peptide is removed, probably at the ER stage. Efficient phosphate-repressible expression of both toxin and immunity still occurs. Nuclear gene mutants affecting processing and secretion of the precursor (KEX, REX and SEC), have been employed to define the pathway of toxin and immunity biosynthesis, and protein fusions to E.coli β-galactosidase have been used to identify the immunity determinant.

1501 GENE FUSIONS TO STUDY INTRACELLULAR ASPECTS OF PROTEIN DELIVERY, Scott D. Emr, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The protein constituents of the various subcellular organelles in yeast are initially synthesized in the cytoplasm prior to being directed to their proper subcellular destinations. Gene fusions are being used to map the targeting information present in a yeast vacuole enzyme, carboxypeptidase Y (CPY), and a mitochondrial protein, the F_1-ATPase β-subunit, that participate in the accurate segregation of these proteins into their respective organelles. This approach has demonstrated that short amino-terminal sequence domains on each of these proteins contain sufficient information to direct proper delivery of the proteins. CPY sequences when fused to the normally secreted enzyme invertase, direct this sucrose cleaving enzyme to the vacuole via the secretory pathway. Mutants defective in this delivery process have been isolated. In the mutants, the CPY-Invertase hybrid protein as well as wild-type CPY protein (also expressed in these strains) are misdirected to the cell surface. The mutants should help to define nuclear genes whose products participate in vacuole assembly. In an analogous approach, when β-subunit amino-terminal sequences are fused either to *E. coli* β-galactosidase or to yeast invertase, they direct the import of these enzymes into yeast mitochondria. Mutants have been isolated that affect import of the hybrid proteins. Some of the mutants exhibit a temperature conditional growth defect. At the nonpermissive temperature, import of normal β-subunit protein is impaired. Further characterization of these mutants should help define some of the cellular components that are required for the assembly of functional mitochondria.

1502 COORDINATE REGULATION OF PHOSPHOLIPID SYNTHESIS IN YEAST, Susan A. Henry, Department of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461.

The synthesis of a number of phospholipids of the yeast membrane is subject to coordinat regulation(1). The enzymes under coordinate control include the cytoplasmic enzyme, inositol-1-phosphate synthase (I-1-PS) which mediates a key step in the biosynthesis of inositol, an important phospholipid precursor. In addition, the regulation encompasses a series of membrane associated enzymes which mediate the reaction series starting with the synthesis of phosphatidylserine (PS) from cytidine diphosphate diacyclglycerol (CDP-DG) and free serine and culminating in the synthesis of phosphatidylcholine (PC). The coordinately regulated membrane associated enzymes include phosphatidylserine synthase (2), phosphatidylserine decarboxylase (3) and the phospholipid-N-methyltransferases which convert phosphatidyl-ethanolamine (PE) to PC (4). Indeed the complete reaction series; CDP-DG→PS→PE→→→PC; appears to be coordinately regulated (1).

Phosphatidylserine synthase and the phospholipid-N-methyltransferases are repressed by the simultaneous addition of inositol and choline (1,4,2,5), whereas I-1-PS is repressed by the addition of inositol alone. However, I-1-PS is thought to be subject to the same coordinate regulation which controls the other enzymes. A series of regulatory mutants isolated on the basis of their effects upon I-1-PS have now been shown to have similar effects upon expression of the coordinately regulated membrane associated enzymes. These mutants include the *ino2* and *ino4* mutants which are unable to derepress the coordinately regulated activities and the *opi1* mutant, which expresses all of the activities constitutively. In addition, a new class of mutants (*opi5*) has been isolated which uncouples the expression of I-1-PS from the coordinate regulation. The epistatic interactions of all of the regulatory genes is presently being established. Two of the genes encoding coordinately regulated activities (*INO1*, structural gene for I-1-PS and *CHO1*, structural gene for phosphatidylserine synthase) have recently been cloned and the *INO4* regulatory gene has also been isolated. The availability of these genes as probes for molecular studies will permit a detailed analysis of this complex regulation.

1. Henry, S.A., Klig, L.S. and Loewy, B.S. 1984. Ann. Rev. Genetics. In Press.
2. Carson, M.A., Atkinson, K.D. and Waechter, C.J. 1982. Biol. Chem. *257*: 8115.
3. Carson, M.A. *et al.*, 1984. Biol. Chem. *259*: 6267.
4. Waechter, C.J. and Lester, R.L. 1973. Arch. Biochem. Biophys. *158*: 401.
5. Yamishita, S. *et al.*, 1982. Eur. J. Biochem. *128*: 589.

1503 ISOLATION OF THE PUTATIVE CLATHRIN HEAVY CHAIN GENE FROM S. CEREVISIAE, Gregory S. Payne and Randy Schekman, Department of Biochemistry, University of California, Berkeley, CA 94720

Newly synthesized proteins destined for the plasma membrane or secretion traverse a series of membrane-enclosed organelles which comprise the secretory pathway. A crucial aspect of transport through this pathway involves vesiculation of secretory organelle membranes to generate transport vesicles capable of fusing with subsequent organelles. Coated membranes and vesicles have been implicated as intermediates in the process of vesicle formation. In mammalian cells the lattice structure which coats membranes is principally composed of an 180kd clathrin heavy chain and two light chains of approximately 35kd. In order to test more precisely the role of clathrin in intracellular transport, we have cloned the gene for the large subunit with the intention of producing mutations in the chromosomal locus. Using a modification of the procedure described by Mueller and Branton (1) we have isolated from S. cerevisiae cells a membrane fraction enriched for coated vesicles. The protein profile of this fraction displays a prominent 190kd polypeptide and a 36kd polypeptide. Treatment with either 2M urea or 0.5M Tris dissociates the 190kd and 36kd proteins from the vesicles. These two proteins, released from vesicles in equimolar amounts by 2M urea, cofractionate when chromatographed through Sepharose 4B. This distinctive behavior and subunit ratio is analogous to that of clathrin heavy and light chain components of mammalian coated vesicles. We have generated a polyclonal rabbit antiserum which specifically recognizes the 190kd putative yeast clathrin heavy chain. The antiserum has been employed in an immunological screen of a library of yeast DNA inserted into bacteriophage λgt11 (2). One unique recombinant bacteriophage was found that expresses an immunoreactive protein. Extracts prepared from cells infected with the recombinant bacteriophage, when coupled to nitrocellulose, can deplete the antiserum of antibodies which recognize the 190kd protein. Antibodies absorbed by the nitrocellulose-bound extract can be recovered and specifically detect the 190kd polypeptide in extracts prepared from yeast cells. Lysates of cells infected with the λgt11 vector do not absorb antibodies specific for this protein. Furthermore, probe synthesized from the yeast DNA hybridizes to a single 5.5kb polyadenylated RNA species. This RNA is large enough to code for the 190kd protein.

(1) Mueller, S. C. and D. Branton. 1984. J. Cell. Biol. 98:341.
(2) Young, R. A. and R. W. Davis. 1983. Proc. Natl. Acad. Sci. USA 80:1194.

1504 ENDOCYTOSIS IN YEAST, Howard Riezman, Yolande Chvatchko and Isabelle Howald, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland.

Uptake of fluid by endocytosis can be followed in *Saccharomyces cerevisiae* cells using a fluorescent dye, lucifer yellow CH. Uptake of the dye is time, temperature and energy dependent and is not saturable. Internalized lucifer yellow is accumulated in the vacuole. Many of the yeast mutants conditionally defective in secretion (1) are also defective in endocytosis. Analysis of these mutants leads to the following conclusions. Efficient transport of proteins from the endoplasmic reticulum to the Golgi apparatus and from the Golgi apparatus to secretory vesicles is not necessary for endocytosis. Part of the endocytic pathway may be obligatorily coupled to the late stages of secretion. We are isolating mutants which are defective in endocytosis but not in secretion. At least one such mutant will be described.

1. Novick, P., Fields, C. and Schekman, R. (1980) Cell 21, 205-215.

1505 CHARACTERIZATION OF YEAST MUTANTS IN ASPARAGINE-LINKED GLYCOSYLATION, *Phillips W. Robbins, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139*

We believe that the characterization of mutants will be useful in unraveling the pathway of asparagine-linked glycosylation and have been working with the yeast Saccharomyces cerevisiae. While recent studies with mammalian cell mutants have made valuable contributions to the understanding of protein glycosylation, the relative ease of obtaining mutants in yeast has allowed us to use a genetic and biochemical approach which has been less feasible in animal cell systems. Like higher eukaryotic cells, yeast make a lipid-linked oligosaccharide $Glc_3Man_9GlcNAc_2$ which is transferred en bloc to protein and then modified by excision of the glucose residues. While further processing in animal cells can involve the removal of up to six mannose residues and addition of sugars such as N-acetylglucosamine, galactose, fucose, and sialic acid, yeast cells add mannose residues and produce oligosaccharides ranging in size from 13 mannose residues, in the case of carboxypeptidase Y to over 50, in the case of invertase.

We have used a [^{3}H]-mannose suicide selection to enrich for mutants in lipid-linked oligosaccharide biosynthesis (see Huffaker and Robbins, J. Biol. Chem. 257: 3203-3210, 1982). Six complementation groups were isolated, each accumulating a different type of lipid-linked oligosaccharide in vivo at 36°C: alg1 accumulates $GlcNAc_2$; alg2 accumulates $Man_2GlcNAc_2$; alg3 accumulates $Man_5GlcNAc_2$; alg4 accumulates a mixture of oligosaccharides including $Man_9GlcNAc_2$, $Man_7GlcNAc_2$ and $Man_5GlcNAc_2$; alg5 and alg6 both accumulate $Man_9GlcNAc_2$. The characterization of these complementation groups will be discussed.

1506 PROTEOLYTIC PROCESSING OF THE MATING PHEROMONE PRECURSOR, PREPRO-α-FACTOR. Jeremy Thorner[1], Robert S. Fuller[1], Anthony J. Brake[2] and David J. Julius[1], [1]Department of Microbiology and Immunology, University of California, Berkeley, CA 94720 and [2]Chiron Research Laboratories, Chiron Corporation, Emeryville, CA 94608.

S. cerevisiae secretes two biologically-active peptides, the mating pheromone α-factor and killer toxin. We have demonstrated that α-factor is initially synthesized as a substantially larger glycosylated precursor protein, prepro-α-factor (1,2,3). These studies and examination of the organization of the α-factor structural genes (MFα1 and MFα2) by ourselves (2) and others (4,5) provided strong suggestive evidence that the first proteolytic processing event in α-factor maturation is cleavage at pairs of basic residues (-Lys-Arg-) that flank the mature pheromone sequences within the precursor. Killer toxin is also derived by excision from a larger precursor that contains pairs of basic residues at several presumptive processing sites (6). A mutation (kex2) was identified previously that pleiotropically blocked the production of active α-factor and the release of functional killer toxin. We have shown that prepro-α-factor is not proteolytically processed at all in kex2 mutants and that the absence of a novel endopeptidase specific for cleaving on the carboxyl side of pairs of basic residues is responsible for the observed processing defect (7). We cloned the KEX2 gene from a yeast genomic library (7) and have utilized the cloned gene to over-produce its protein product to permit detailed characterization of its structure, catalytic properties, and subcellular localization. The KEX2 enzyme is a neutral, membrane-bound, thiol protease, with an apparent native MW of 105,000 after solubilization using the detergent deoxycholate. Remarkably, enzyme activity and covalent labeling of the protein by an affinity reagent, [^{125}I]Tyr-Ala-Lys-Arg-chloromethylketone, require Ca^{++} ion. After inactivation by EDTA treatment, reactivation of the enzyme can be achieved at a free [Ca^{++}] of micromolar or less. In these respects and others, the yeast KEX2 enzyme resembles a class of Ca^{++}-dependent mammalian proteases called "calpains". The predicted primary structure of the KEX2 protein deduced from the DNA sequence of the gene is informative in light of the properties of the enzyme. There are two strikingly hydrophobic regions: a 22-residue signal sequence at the N-terminus and a 21-residue potential membrane-spanning domain about 70 residues from the C-terminus. There are five potential sites for the addition of N-linked oligosaccharide and a strikingly serine- and threonine-rich region just to the amino-terminal side of the membrane-spanning segment that may provide sites for the addition of O-linked oligosaccharide.

(1) Julius, D. et al Cell 32: 839-852 (1983); (2) Brake, A. et al Molec. Cellul. Biol. 3: 1440-1450 (1983); (3) Julius et al Cell 36: 309-318 (1984); (4) Kurjan, J. and Herskowitz, I. Cell 30: 933-943 (1982); (5) Singh, A. et al Nucl. Acid. Res. 11: 4049-4063 (1983); (6) Bostian, K. et al Cell 36: 741-751 (1984); (7) Julius, D. et al Cell 37: 1075-1089 (1984).

Protein Localization

1507 STUDIES ON THE MECHANISM OF NUCLEAR PROTEIN LOCALIZATION IN YEAST, Michael N. Hall, Dept. Biochemistry & Biophysics, UCSF, San Francisco, CA 94143

In order to identify determinants governing nuclear protein localization, we have constructed a set of gene fusions coding for hybrid proteins containing varying amounts of the yeast nuclear protein α2 (a DNA-binding, regulatory protein) at the amino terminus and a constant, active portion of β-galactosidase (LacZ) at the carboxy terminus. (*Targeting of E. coli β-galactosidase to the nucleus in yeast.* Cell 36, 1057-1065, 1984) The properties of these hybrid proteins are summarized in the following table.

HYBRID	α2 AA's	NUCLEAR LOCALIZATION	BINDS DNA	LETHAL TO HOST WHEN OVERPRODUCED
3α2-LacZ	3	-	-	-
13α2-LacZ	13	+	n.d.	+
25α2-LacZ	25	+	-	+
67α2-LacZ	67	+	-	-
210α2-LacZ	210	+	+	-

The above results argue that nuclear protein localization is not by passive diffusion through nuclear pores with subsequent retention by binding to a non-diffusible intra-nuclear substrate. Proteins may be selectively translocated across the nuclear envelope. I am selecting mutants to identify presumed components of a selective translocation apparatus in the nuclear envelope.

1508 TRANSPORT OF YEAST H2B INTO THE NUCLEUS. Robert B. Moreland, and Lynna M. Hereford, Dana-Farber Cancer Institute and The Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

The sequences required for transport of yeast H2B-1 into the nucleus have been determined using H2B β-galactosidase fusions. Progressive deletions from the carboxyl terminus have revealed that the sequences required for nuclear localization lie between amino acids 28 and 33. The amino acid sequence within this region, LYS_{30} LYS ARG SER LYS_{34} is very similar to the sequence which Kalderon, et.al. (1) have shown to be required for transport of SV40 T antigen. We therefore, constructed a point mutant at H2B LYS_{31}, since an equivalent mutation in T antigen (at LYS_{128}) totally obliterates transport. The H2B point mutant also obliterates transport. Interestingly, the sequences which we have identified as essential for transport lie within a region which Wallis, et.al. (2) have shown to be dispensable. We are testing whether this discrepancy can be explained by cotransport of H2A-H2B, the signal for transport being supplied by the amino terminus of either H2A or H2B.

1. Kalderon, D., Richardson, W.D., Markham, A.F., and A.E. Smith. Nature. 311;33-37.

2. Wallis, J.W., Rykowski, M., and Grunstein, M. Cell. 35;711-719.

1509 NUCLEAR ENTRY OF A PROCARYOTIC PROTEIN IN SACCHAROMYCES CEREVISIAE, Jasper Rine and Georgiana Barnes, Department of Biochemistry, University of California, Berkeley, California, 94720

In eucaryotic cells, the compartmentalization of functions imposes a stringent requirement for proteins to be targeted and localized to an appropriate subcellular location. The best understood examples of localization involve signals in secreted proteins that are recognized by a cellular apparatus that directs the protein toward its destination. Much less is known about the path leading to the nucleus. In a few cases, however, specific regions in certain nuclear proteins have been implicated as the signal for nuclear accumulation of these proteins (e.g. 1). The presence of a signal implies the existence of an apparatus that recognizes the signal, although no such apparatus has been identified for nuclear proteins.

We wished to determine whether a protein that is synthesized in vivo requires a signal for entry into the nucleus. Furthermore, we wanted to set up a genetic selection that could allow the isolation of mutations that block the entry of proteins into the the nucleus. As a step towards both goals, we have constructed gene fusions that allow for the regulated production of the procaryotic protein EcoRI endonuclease in yeast cells. We have shown that the endonuclease does enter the nucleus with relatively rapid kinetics. As a result, the cells die. rad52 mutants, which are deficient in the repair of double strand breaks in DNA, are more sensitive to the effects of the endonuclease than are RAD52 cells. In rad52 mutants, the endonuclease is capable of a complete digestion of the yeast genome at all EcoRI sites and only at EcoRI sites. From these studies, we conclude that a nuclear localization signal is not required for the nuclear entry of a protein. Furthermore, the lethality of EcoRI endonuclease may prove useful in isolating mutations that block the entry of at least some proteins into the nucleus.

Experiments with other organisms suggests that proteins under a certain size may enter the nucleus by a nonspecific means. We have explored the possibility of increasing the effective size of the endonuclease so that its entry into the nucleus will become dependent on a nuclear signal.Specifically,we have demonstrated that it is possible to add amino acids to the amino terminus of the endonuclease in such a way as to retain enzyme activity. The construction and characterization of fusion proteins between the endonuclease and bona fide yeast nuclear proteins is underway.

1. D. Kalderon, W.D. Richardson, A.F. Markham, and A.E. Smith (1984) Nature, 311: 33-38

1510 MUTATIONS AFFECTING LOCALIZATION OF NUCLEAR PROTEINS, Pamela Silver, Department of Biochemistry & Molecular Biology, Harvard University, Cambridge, MA 02138

I have begun to use a genetic approach to understand the problem of nuclear protein localization. I have defined amino acid sequences in a yeast nuclear protein that are sufficient for its accumulation in the nucleus. Specifically, I have made gene fusions between the yeast GAL4 gene and the E.coli lacZ gene. The resulting gene fusions, when placed in yeast, yield chimeric proteins with β-galactosidase activity. The GAL4 gene product, a positive activator protein of the yeast genes necessary for galactose metabolism, is thought to interact directly with DNA. On the other hand, β-galactosidase is not localized to any intracellular compartment when produced in yeast. When portions of the GAL4 N-terminus are fused to β-galactosidase, the β-galactosidase is detected predominantly in the cell nucleus by indirect immunofluorescence. By this analysis, I determined that only 74 of the total 881 GAL4 amino acids are sufficient for nuclear localization. An unexpected finding is that chimeric proteins lacking the nuclear determinant, which are excluded from the nucleus, are produced at higher levels as determined by β-galactosidase activity. The fact that the nuclear-associated chimeric proteins are present at lower levels than otherwise non-nuclear chimeras may reflect some fundamental aspect of the localization process. I have taken advantage of this observation to screen for yeast that produce higher levels of a normally nuclear-associated GAL4-lacZ chimeric protein. I have isolated both cellular and GAL4-linked mutants that display altered localization of the normally nuclear-associated fusion protein.

1511 PROTEIN SORTING AND VACUOLE ASSEMBLY IN YEAST, Tom H. Stevens, Elizabeth G. Blachly, Craig P. Hunter, Carli C. Lam, Joel H. Rothman and Luis A. Valls, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The process of secretion and organelle assembly in yeast is strikingly similar to that found in more complex eukaryotes. Secretory proteins as well as proteins destined for the lysosome-like vacuole are synthesized on ER-bound ribosomes. Both classes of proteins are then transported to the Golgi complex where sorting appears to occur (1). To investigate the mechanisms of sorting and vacuole assembly in yeast we are isolating and biochemically analyzing mutants that fail to correctly localize vacuolar glycoproteins.

The vacuolar glycoprotein carboxypeptidase Y (CPY) is one of a number of vacuolar proteins that transit the secretory pathway as inactive zymogens (2). The CPY precursor (proCPY) contains an 11aa N-terminal extension of the mature CPY, as revealed by DNA sequencing of its structural gene PRC1. The first 20 amino acids of the N-terminal propeptide constitute a hydrophobic signal sequence. However, we have not yet determined whether this signal sequence is actually cleaved upon translocation. The remainder of the propeptide may function to address proCPY to the vacuole. To test this hypothesis we have constructed point and deletion mutations in PRC1 that may result in mislocalization of proCPY.

To investigate the cellular apparatus that recognizes proCPY as a vacuolar protein we have developed a selection scheme which facilitates the isolation of sorting mutants which mislocalize CPY to the periplasm. These putative sorting mutants have been classified into multiple complementation groups, many of which contain temperature-sensitive (ts) alleles. These mutants (vpl, vacuole protein localization) mislocalize a number of vacuolar proteins besides CPY to the cell surface. The pleiotropy of several of these mutations presumably reflects the existence of a common essential sorting pathway used by the cell to localize vacuolar proteins.

To define the vacuole assembly pathway after the branch point from the secretory pathway, random ts mutants were screened for a block in transport specific to vacuolar proteins. We found six mutants (vac) that accumulate proCPY and proproteinase A intracellularly at 37°C even though secretion of normal secretory proteins is not affected. These vac mutants appear to be blocked in transport of vacuolar proteins after the "sorting point" but before the proteolytic processing compartment (presumably the vacuole).

(1) Stevens, T.H., B. Esmon and R. Schekman (1982) Cell 30, 439-448.
(2) Jones, E.W. (1984) Ann. Rev. Genet. 18, 233-270.

1512 MOLECULAR AND GENETIC ANALYSIS OF MITOCHONDRIAL PROTEIN IMPORT. Michael P. Yaffe

A major facet of mitochondrial assembly is the import of most mitochondrial polypeptides from their site of synthesis in the extramitochondrial cytoplasm to their functional location in the organelle. Many of these imported proteins are made as precursors of larger molecular weight, and their import involves: 1) binding of precursor polypeptides to the mitochondrial outer membrane, 2) translocation across one or both mitochondrial membranes, 3) proteolytic processing of larger molecular weight precursors to their mature size, and 4) assembly of subunits into functional enzyme complexes. The role of the amino-terminal presequences, found on many mitochondrial precursors, has been examined by constructing fusions between portions of cloned genes for several mitochondrial proteins and the gene for a cytoplasmic enzyme, dihydrofolate reductase (DHFR). Polypeptides encoded by these gene fusions were synthesized in an in vitro transcription-translation system and incubated with isolated mitochondria. A polypeptide containing only a portion of the pre-sequence of a mitochondrial protein fused to DHFR was imported into the mitochondrial matrix. Studies with this and other constructions indicate that the presequence is sufficient for correct targeting and import of a polypeptide in vitro, and that these sequences also contain information for intramitochondrial targeting. The import of these fusion proteins is also being examined in the intact cell. A second focus of our research has been a characterization of the molecular components of the import apparatus by analyzing temperature-sensitive yeast strains blocked in mitochondrial protein import (mas mutants). Import of proteins into mitochondria isolated from one of these strains (mas 1) is substantially slower than with wild-type mitochondria, while the rate and efficiency of oxidative phosphorylation are essentially identical in the mutant and wild-type mitochondria. The defective components of the import apparatus in mas 1 and other mutant strains are being characterized further by cloning the genes for these components. Availability of these genes should help in identifying the intracellular location and function of molecules mediating the import process.

Protein Localization and Secretion

1513 PHOSPHATIDYLETHANOLAMINE INFLUENCE ON MAINTENANCE OF CENTROMERE-LESS YEAST REPLICON
Katharine D. Atkinson, University of California, Riverside CA 92521

Yeast mutants with defects in biosynthesis of the nitrogenous phospholipids exhibit mitotic instability of the mitochondrial genome and of centromere-less circular plasmids. Comparison of several mutants specifically implicates phosphatidylethanolamine deficiency as the cause of extrachromosomal genome instability.

Yeast chol mutants require either choline or ethanolamine because they cannot make phosphatidylserine (PS), the precursor of phosphatidylethanolamine (PE) and phosphatidylcholine. Recessive suppressors, altered at eam1, eam2, or eam3, make endogenous ethanolamine, feeding chol mutants from within. The chol eam strains, lacking PS but sustaining normal PE levels, maintain extrachromosomal elements normally. The un-suppressed chol mutants, lacking PS and deficient in PE when fed choline, show maintenance instabilities: a) they lose their mitochondrial genomes, with a 50 to 90% incidence of ^{0} respiration-defective segregation, b) they do not exhibit chromosomal instability, yielding a low incidence of copper-resistant chromosome VIII aneuploids, c) they are transformed with normal efficiency by the CEN^{+} plasmid YCp19, but d) they are transformed only 1% as efficiently by the related centromere-less YRp7 and YRp12 plasmids. This indicates that establishment of transforming plasmid DNA is affected, but uptake is not. Normal CHO^{+} EAM^{+} strains also transform more efficiently in a Li^{+} promoted system when their membrane PE composition is augmented by ethanolamine supplementation.

1514 ASSEMBLY OF THE CYTOCHROME B-C_1 COMPLEX IN THE YEAST MITOCHONDRIAL MEMBRANE, Diana S. Beattie and Keya Sen, Department of Biochemistry, Mount Sinai School of Medicine, New York, N.Y. 10029

The cytochrome b-c_1 complex of the mitochondrial respiratory chain catalyzes electron transport coupled to ATP synthesis from reduced coenzyme Q to cytochrome c. An enzymatically active b-c_1 complex isolated from bakers' yeast contains 7-8 subunits ranging in molecular weight from 49-11 kDa. The iron-sulfur protein (ISP) is synthesized in vivo in the cytosol as a larger precursor polypeptide of 28 kDa. The precursor is subsequently cleaved, in a process requiring an energized mitochondrial inner membrane, into an intermediate form 1.5 kDa larger than the mature subunit (24 kDa). The conversion of the intermediate to the mature form occurs in the inner mitochondrial membrane while it is associated with the other proteins of the complex. In mutants lacking cytochrome b, the mitochondrial membranes contain 50% or less of the ISP determined by immuno-blotting or immunoprecipitation with specific antisera, although cytochrome c_1 is present in the same amount as the wild type. Kinetic studies on the labeling of ISP indicated that the precursor form of this protein is synthesized in the cytochrome b lacking cells but that the subsequent processing and/or assembly into the membrane is affected. These results are consistent with our previous suggestion (JBC 258, 19649, 1983) that cytochromes b and c_1 which are rapidly synthesized in vivo may act as a template for the subsequent insertion of the other subunits of the complex into the membrane.

1515 ANALYSIS OF THE YEAST SEC53 GENE AND GENE PRODUCT, Mitchell Bernstein[1], Gustav Ammerer[2], Werner Hoffmann[2], and Randy Schekman[1], [1]Department of Biochemistry, University of California, Berkeley, CA 94720; [2]ZymoGenetics, 2121 N. 35th St., Seattle, WA 98103.

We are studying SEC53, a gene involved in movement of proteins through the early part of the secretory pathway in S. cerevisiae. Mutations in this gene cause inactive, unglycosylated invertase to accumulate, bound to the membrane, in the lumen of the endoplasmic reticulum. Plasmids that complement sec53 have been isolated from genomic and cDNA libraries. We have shown that these plasmids contain the SEC53 gene by integration of sequences into the genome, followed by genetic analysis. We have also shown SEC53 to be essential for cell growth by gene disruption experiments.

The SEC53 gene is transcribed into an mRNA of approximately 1000 nucleotides, accounting for 0.1%-0.5% of the yeast poly A+ RNA. We have also sequenced SEC53 and found an open reading frame capable of encoding a 28 kD polypeptide. When SEC53 is present in cells on a multicopy plasmid, a protein of this molecular weight is overproduced. This protein has been identified unequivocally as the SEC53 gene product (Sec53p) through a combination of transposon mediated mutagenesis of plasmid DNA, and hybrid selection of yeast RNA followed by in vitro translation.

We have made a gene fusion of SEC53 to LACZ, and have used the resultant hybrid protein, purified from E. coli, to raise a polyclonal antiserum against Sec53p. We are currently fractionating cells to determine the cellular location of Sec53p, and conducting in vitro experiments to further elucidate the nature of the sec53 defect.

1516 Extragenic Suppressors of Signal Sequence Mutations in Yeast. Elizabeth G. Blachly and Tom H. Stevens, University of Oregon, Eugene, OR 97403.

Translocation of proteins across the endoplasmic reticulum (ER) membrane has been studied intensively in animal cells. This work has led to the discovery of the signal recognition particle (SRP) and the SRP receptor. We are using a genetic approach to analyze the molecular details of this highly conserved translocation process in the yeast S. cerevisiae.

The DNA sequence of PRC1, the structural gene for carboxypeptidase Y (CPY), indicates that this vacuolar glycoprotein is initially synthesized with an N-terminal signal sequence that contains a 16 amino acid hydrophobic core. We have produced a series of deletion mutations (Δssl-4) that reduce the length of the hydrophobic core. The mutant polypeptides are not translocated across the ER membrane and remain in the cytoplasm as inactive, unglycosylated precursors.

To obtain mutations in genes coding for components of the translocation machinery that recognize and bind signal sequences, we have selected unlinked suppressors of the prc1 signal sequence mutations. Mutants were obtained by selecting for CPY activity in a prc1-Δssl strain, using a selection scheme developed in our laboratory. We have obtained extragenic suppressors which allow translocation of signal sequence defective CPY across the ER membrane. A number of these mutations produce a ts lethal phenotype. Biochemical and complementation analyses of these mutants will be presented.

1517 MOLECULAR ANALYSIS OF A MITOCHONDRIALLY-IMPORTED ENZYME INVOLVED IN PROLINE UTILIZATION IN YEAST, Marjorie C. Brandriss and Karen A. Krzywicki, Department of Microbiology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

The enzyme Δ^1-pyrroline-5-carboxylate (P5C) dehydrogenase participates in the conversion of proline to glutamate inside the mitochondria of Saccharomyces cerevisiae. The protein is synthesized in the cytoplasm as a precusor of 64 kd molecular weight. During import into the mitochondria, it is processed to its mature form by the removal of 15-20 amino acids. We are interested in learning more about the import process and have begun to introduce changes in the protein by altering the cloned gene. First, we have made gene fusions between lacZ (ß-galactosidase) and PUT2 (P5C dehydrogenase). A protein fusion carrying the amino-terminal 14 residues of P5C dehydrogenase attached to ß-galactosidase was not targeted to the mitochondria and was found exclusively in the cytoplasm. A hybrid ß-galactosidase protein carrying the first 366 residues of P5C dehydrogenase was localized inside the mitochondria and, when made in large amounts, had deleterious effects on cell growth. Additional put2-lacZ fusions are under construction to delimit the sequences important for targeting ß-galactosidase to the mitochondria. Second, we constructed an internal in-frame deletion of the PUT2 gene that removed DNA between codons 14 and 124. This resulted in the formation of a P5C dehydrogenase protein that was not imported into the mitochondria in an in vitro import assay. The aim of these studies is to determine which sequences or conformations of P5C dehydrogenase are important in directing the protein to the mitochondrial membrane and in finding its ultimate position within that organelle.

1518 REGULATION OF HO GENE EXPRESSION, Linda Breeden and Kim Nasmyth, MRC Laboratory of Molecular Biology, Cambridge, U.K.

The diploidization of Saccharomyces cerevisiae is achieved by a very specific pattern of mating type switching, which involves a double strand break at the MAT locus by the HO endonuclease. The pattern of switching may be explained by the pattern of HO transcription, which occurs only during late G_1 in a or α mother cells, and is absent throughout the cell cycle of a or α daughter cells and a/α diploids.

In order to determine how this cell type- and cell cycle-specific expression of HO is accomplished we have carried out an extensive deletion analysis to identify cis acting regulatory elements at HO. Sequences more than a kilobase upstream from HO are required for HO transcription. In between this region (URS1) and the TATA region, there are about ten copies of a short sequence (CACGAAAA) that appear to be involved in limiting HO expression to late G_1 in the cell cycle.

Studies are underway to identify the genes whose products act in trans on $CACGA_4$ sequences.

1519 **INVOLVEMENT OF THE WALL-MEMBRANE COMPLEX IN DNA TRANSFORMATION.** Carlo V. Bruschi and Gregg A. Howe, Department of Microbiology - School of Medicine and Department of Biology, East Carolina University, Greenville, NC 27834-4354.
The role of the wall-membrane complex (WMC) in DNA transformation has been investigated, and a new, more efficient method of DNA transformation of whole yeast cells with $CaCl_2$ has been developed. Transformation efficiency is more than 1,000 transformants /µg DNA and varies with the type and size of the plasmids. The methodological approach has been to study the effect of several parameters related to the biology of the WMC on the transformation efficiency. Such parameters are: growth phase, extracellular concentration of Ca^{++} ions, heath shock, presence of the transforming DNA at various steps of the transformation process, sensitivity to permeabilizing agents, osmotic stability and physiological recovery from permeabilization. Irreversible adsorbtion of the plasmid DNA molecules to the WMC is induced by both, heath shock and polyethyleneglycol. Cotransformation with two and three vectors at the time has been analyzed in order to establish whether or not the WMC expresses any selectivity towards a particular DNA molecule. The presence of the transforming plasmids within the cell has been demonstrated at the molecular level by gel electrophoresis and Southern hybridization. High percentage of mitotic loss demonstrates a non-mendelian way of inheritance of the plasmids, excluding the possibility of integration or of mitotic gene conversion (2).
(2) Carlo V. Bruschi and Gregg A. Howe (1984). XIIth International Conference on Yeast Genetics and Molecular Biology, Abstract P-19, p. 286.

1520 A MUTATION IN THE C-TERMINAL 2/3 OF A MITOCHONDRIALLY ENCODED PROTEIN AFFECTS BOTH PROCESSING OF THE LEADER PEPTIDE AND PROTEIN FUNCTION, Vicki L. Cameron, Joan E. McEwen, and Robert O. Poyton, University of Colorado, Boulder, CO 80309

Subunit II of cytochrome c oxidase in yeast is the only protein encoded on mitochondrial DNA that is initially synthesized as a precursor with a leader peptide at its N-terminus. In an effort to understand the function of this leader peptide, we have generated, by use of a new method, a large number of mutations in the subunit II gene. Twenty-four strains carrying new mutations in the subunit II gene have been characterized. By crossing to a rho- petite which contains only a portion of the gene, we have mapped five of these mutants to the amino-terminal 1/3 of the gene; the rest map to carboxyl-terminal 2/3 of the gene. Of particular interest for analysis of leader peptide processing and function is a mutant, VC21, that appears to accumulate the precursor to subunit II. The mutation is a T to A change at nucleotide 370. The nucleotide change results in substitution of an arginine for a tryptophan at amino acid 124 in the protein sequence. A model for how such a change, almost 1/2 way through the protein, could affect leader processing and protein function is presented.

1521 α-FACTOR DIRECTED EXPRESSION AND SECRETION OF E. COLI β-GALACTOSIDASE IN YEAST, SACCAROMYCES CEREVISIAE. Rathin C. Das and Janice L. Shultz, Biosynthesis Research, Miles Laboratories, Inc., Elkhart, IN 46515

Yeast pheromone α-factor is a 13 residues long peptide secreted into the growth medium by the α-mating type cells of yeast, Saccharomyces cerevisiae. It has been shown recently that α-factor is synthesized initially as a larger precursor polypeptide, prepro-α-factor. The precursor contains four copies of the mature pheromone within it and undergoes a number of posttranslational modifications before the mature and biologically active α-factor is secreted out of the cell. In order to understand the detailed molecular mechanism of protein expression, processing and secretion in yeast, we have used the prepro leader sequence of the α-factor gene to express E. coli β-galactosidase in yeast.

Experiments with yeast α and a cells harboring the prepro-α-factor-β-galactosidase fused gene under the control of the α-factor promoter indicate a very high level of β-galactosidase activity in α-cells as compared to a cells. This gene fusion approach therefore, should permit a detailed study of the intracellular transport and secretion of E. coli β-galactosidase in yeast. Details of the construction of the fused gene, regulation of the expressed protein and analysis of protein localization will be discussed.

1522 ALPHA SUBUNIT OF THE ATP SYNTHETASE IS MITOCHONDRIALLY MADE AND ENCODED IN THE YEAST-LIKE PROTOTHECA ZOPFII, M. Winnann Ewing and Donald Deters, University of Texas, Austin, Texas 78712-1095.

P. zopfii is a unicellular, achloric, heterotrophic yeast-like organism which is classified as an alga based on its method of spore production. Mitochondrial DNA from this organism was isolated, analyzed by digestion with various restriction enzymes and found to have a size of approximately 50 kb. Southern hybridization, using the nuclearly encoded gene for the alpha subunit of the ATP synthetase from Saccharomyces cerevisiae as a probe, indicates that the P. zopfii gene for the alpha subunit of ATP synthetase is present in mtDNA. In vivo labeled mitochondrial translation products include a 60,000 dalton protein which copurifies with mitochondria on Percoll gradients. Isolation of the ATP synthetase from this organism, as expected, yields a complex made up of several polypeptides, the largest (alpha) of which comigrates with the labeled band on SDS polyacrylamide gels. Limited proteolysis, using StaphV8 protease or papain, results in essentially identical digestion patterns for the labeled peptide and the authentic alpha subunit. These results indicate that in P. zopfii the gene for the alpha subunit is present on mtDNA, and that the protein is, in fact, mitochondrially made. Supported in part by grant F925 from The Robert A. Welch Foundation.

1523 Identification of a Nuclear Transport Signal of Yeast Ribosomal Protein L3. Howard M. Fried, Robert B. Moreland, and Lynna Hereford, University of North Carolina, Chapel Hill, NC 27514 and Dana-Farber Cancer Institute, Boston, MA 02115.

In eukaryotic cells, newly synthesized ribosomal proteins rapidly enter the nucleus where they assemble with rRNA into pre-ribosomal subunits. As a first step to determining how this transport is accomplished, we have used the technique of gene fusion to identify a region of a ribosomal protein which targets it to the nucleus. Hybrid genes consisting of amino terminal segments of rpL3 joined to a carboxy terminal segment of *E. coli* β-galactosidase were introduced into yeast. The locations of the hybrid proteins were determined by *in situ* immunofluorescence with anti-β-galactosidase antibody. A segment of the first 21 amino acids of rpL3 was sufficient to direct β-galactosidase to the nucleus. Interestingly, a fusion protein containing all but 14 amino acids from the carboxy terminus of rpL3 was not transported; we discovered, however, that addition of 8 amino acids encoded by 3 tandem oligonucleotide linkers inserted between the rpL3 and β-gal junction produced a protein which was transported. Our results suggest that ribosomal proteins are not co-translationally transported to the nucleus and are consistent with previous failure to find ribosomal proteins synthesized at any specific site in eukaryotic cells. All of the fusion proteins which are transported have detrimental effects on growth, although none are lethal. The 21 amino acid transport domain of rpL3 shares structural homology with analogous domains of other nuclear transported proteins from yeast as well as animal cells.

1524 52 AMINO ACIDS FROM THE AMINO TERMINUS OF THE POLYOMA VIRUS CAPSID PROTEIN VP_1 ARE SUFFICIENT TO LOCALIZE β-GALACTOSIDASE TO THE NUCLEUS IN YEAST, R.L. Garcea, L. Hereford, and R. Moreland, Dana-Farber Cancer Institute, Boston, MA 02115

Protein fusions to bacterial β-galactosidase have become a powerful tool in studying the transport of proteins to the cell nucleus. In an attempt to determine the generality of the yeast nuclear transport system, we have chosen as a model the polyoma capsid protein VP_1, which is transported to the nucleus of mouse cells during lytic viral infection. We have fused 52 amino acids of the VP_1 amino terminus to β-galactosidase, and expressed this fusion protein in yeast using a 2μ vector and the yeast GAL 10 promoter. Upon galactose induction the fusion protein is expressed at levels enabling detection by anti-β-galactosidase antibodies. Indirect immunofluorescence using these antibodies localizes the fusion protein primarily to the cell nucleus. Within the 52 amino acids of VP_1 there is one sequence (Pro Lys Arg Lys Ser) that resembles other known nuclear "signal" sequences. We are presently using Bal-31 nuclease to deletion map the minimal localizing peptide sequence. Localization of this viral fusion protein within the yeast nucleus indicates that the protein signals and cellular pathways for nuclear transport may be in common for many eukaryotes.

1525 GENETIC STUDIES OF YEAST MITOCHONDRIAL PROTEIN IMPORT, Jinnie M. Garrett[1], Mark McCammon[2], Michael G. Douglas[2] and Scott D. Emr[1], [1]Division of Biology, Caltech, Pasadena, CA 91125 and [2]University of Texas, San Antonio, TX 78284

We are studying the mechanism of mitochondrial protein import using gene fusons between the ATP2 gene (nuclear gene coding for the β-subunit of yeast mitochondrial F_1-ATPase) and the *E. coli lacZ* gene. Hybrid proteins containing more than 200 amino acids of β-subunit are efficiently targeted to the mitochondrion (see McCammon et al., op. cit.) and prevent normal functioning of the organelle. Cells expressing these hybrids are no longer able to grow on a non-fermentable carbon source, e.g., glycerol. The Gly^- phenotype has been exploited to select revertants of this defect. Mutants obtained in this selection were screened for normal β-galactosidase levels. Most of the Lac^+ mutants were found to be chromosome-linked, and many are temperature-sensitive on all media tested. Several of the mutants show a defect in import of both the hybrid protein and normal β-subunit. Precursor β-subunit accumulates in such mutant cells.

Two recessive temperature-sensitive mutants obtained in this selection have been further characterized. Transformation of these mutants with a yeast genomic DNA library has given clones that restore the strains to temperature resistance. Further characterization of these clones is now in progress to try and define cellular components which are involved in mitochondrial delivery and import.

1526 **ANALYSIS OF THE STRUCTURE, FUNCTION AND INTRACELLULAR TRANSPORT OF AN INTEGRAL MEMBRANE PROTEIN - THE HAEMAGGLUTININ OF INFLUENZA VIRUS.** M.-J.Gething and J.Sambrook. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.11724, U.S.A.

In recent years, we have used the haemagglutinin (HA) of influenza virus as a model to study eukaryotic integral membrane proteins. Our major interests are two-fold; firstly to correlate structure with function by identifying and analysing the protein domains or epitopes involved in receptor recognition, enzyme activity and antigenicity, and secondly to understand the mechanisms that determine the route of transport and final destination of nascent glycoproteins in eukaryotic cells. HA is the best characterized of all integral membrane proteins: its three-dimensional structure is known and the location of its major antigenic sites, the points at which it is glycosylated, its organization into trimeric structures and its orientation with respect to the membrane have been defined. The cloned gene for HA can be expressed with very high efficiency in mammalian cells, and the protein produced from the wild-type gene is displayed on the cell surface in a glycosylated form that is both biologically and antigenically active. Mutant HAs have been constructed and analysed that are altered in their transport through the cell. Experiments are underway to express the wild-type and mutant HA genes in wild-type and sec strains of yeast.

1527 THE LOCALIZATION OF CHITIN SYNTHETASE IN SECRETORY MUTANTS OF YEAST, Barbara A. Hanson, University of Kentucky, Lexington, KY 40506-0225.

Early studies (P.N.A.S. 72:3952; 80:3318) of chitin synthetase (CS) in yeast suggest it is localized on the plasma membrane. We have found using a new procedure for isolating yeast plasma membranes, that most CS activity was localized in intracellular membranes in an inactive form (85-95%) with only a small amount of activity (10-15%) on the plasma membrane in an active form. The intracellular membranes were separated further on sucrose density gradients into microsomal and light membrane (LM) fractions. The LM fraction contained about 65% of cell CS activity. This activity remained in the supernatant after acid phosphatase and oligomycin-insensitive ATPase activities which are found in secretory vesicles were precipitated by lowering the pH of the fraction to 5.0 and centrifugation. The microsomal fraction, containing the endoplasmic reticulum (ER) and other membranes, had about 24% of the total CS activity. When CS activity was measured in a temperature-sensitive secretory mutant which accumulated secretory vesicles at the nonpermissive temperature (sec-1), there was a 4-fold drop in the plasma membrane fraction and a 2-fold increase in the LM fraction. In the sec-18 mutant, blocked in protein translocation from the ER, the enzyme activity increased 7-fold in those membranes. These results suggest that CS is synthesized in the ER and translocated in membrane structures which are distinct from secretory vesicles in the LM fraction to the plasma membrane where the enzyme is activated.

1528 PROCESSING OF A VIRAL MEMBRANE GLYCOPROTEIN EXPRESSED IN YEAST, SACCHAROMYCES CEREVISIAE, M. Abdul Jabbar, N. Sivasubramanian and Debi P. Nayak, Jonsson Comprehensive Cancer Center, Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

The yeast, Saccharomyces cerevisiae possess a defined secretory pathway capable of processing both secretory as well as integral membrane proteins. Recent studies have shown that heterologous secretory proteins containing signal sequences are processed in S. cerevisiae. In the present investigation, cDNA copy of an influenza viral (WSN/A/33) hemagglutinin (HA) gene coding for an integral membrane glycoprotein was placed under the control of ADH1 promoter of the yeast. The chimeric plasmid is shown to direct the synthesis of hemagglutinin antigens. Moreover, the expressed HA is glycosylated and membrane-bound, suggesting that HA traversed the yeast secretory pathway. These and other results will be presented to discuss the potential use of this system in the development of a subunit vaccine and also to elucidate the steps involved in the transport of a heterologous membrane glycoprotein in yeast.

1529 EXPRESSION OF ANIMAL VIRUS PROTEINS IN *SACCHAROMYCES CEREVISIAE*

Sirkka Keränen and Kai Korpela. Recombinant DNA Laboratory, University of Helsinki, SF-00380 Helsinki, Finland.

cDNA copies encoding the structural proteins of an animal virus, Semliki Forest virus (SFV), were inserted in a yeast expression vector and introduced into *S. cerevisiae* cells. In animal cells the structural proteins of SFV are translated as a polyprotein which is post-translationally cleaved to the final size polypeptides. The N-terminal nucleocapsid protein remains in the cytoplasm while the envelope proteins are inserted into the endoplasmic reticulum membrane and transported to the plasma membrane via Golgi complex. Synthesis and processing of the polyprotein and the fate of the viral polypeptides in the yeast cells will be discussed.

1530 NUCLEAR MITOCHONDRIAL INTERACTIONS: IDENTIFICATION OF THREE NUCLEAR GENES REQUIRED FOR THE POST-TRANSCRIPTIONAL PROCESSING OF A MITOCHONDRIAL GENE, OXI-2.

Barbara Kloeckener-Gruissem, Joan McEwen and Robert O. Poyton, University of Colorado, Boulder, CO 80309.

The functional enzyme complex cytochrome c oxidase requires correct assembly of 9 non-identical subunits in the inner-mitochondrial membrane. Since these subunits are contributed by two distinct genetic systems, the nuclear-cytoplasmic fraction (subunits IV - VIII) and the mitochondrion (subunits I -III), it seems very likely that communication exists between the nucleus and the organelle. Here, we present evidence for nuclear control of expression of one mitochondrial gene. Synthesis of subunit III, the smallest of the three mitochondrially encoded subunits, requires the action of at least three different nuclear genes. This conclusion is based on genetic and biochemical analysis of 9 independently-derived nuclear cytochrome oxidase deficient mutants, which are assigned to three complementation groups (pet 5, pet 20 and pet 21).

In vivo labeling of the mitochondrial translation products in these mutants shows that all major polypeptides are synthesized with the exception of subunit III. Analysis of the mutant mitochondrial transcripts by northern blot hybridization reveals no detectable difference between the mutant and the wild type cox-2 messenger RNA.

The role and possible models of this nuclear-mitochondrial interaction at the level of mitochondrial post-transcriptional processing will be discussed.

1531 REGULATION OF LIPID-LINKED OLIGOSACCHARIDE ASSEMBLY IN SACCHAROMYCES CEREVISIAE.

M. A. Kukuruzinska, J. R. Couto, and P. W. Robbins; M.I.T.; Cambridge, MA 02139.

The asparagine-linked chains of glycoproteins are derived from a common lipid-linked precursor oligosaccharide, Dol-PP-GlcNAc$_2$Man$_9$Glc$_3$, (LLO), which is synthesized by a stepwise transfer of sugars to the lipid carrier in the membrane of the ER. To date little is known about the regulation of the assembly of the LLO precursor and the overall glycoprotein synthesis. The yeast S. cerevisiae is an excellent model organism since it allows for isolation of mutants which are defective in various steps of LLO assembly. We are investigating the regulation of the LLO pathway by studying the enzymatic activity of the UDP-GlcNAc:Dol-P transferase, the first enzyme in the LLO assembly, and by measuring the levels of mRNA for this enzyme in various mutant backgrounds. Two Ts mutants defective in the asparagine-linked glycosylation, alg1 and alg2, (blocked at Dol-PP-GlcNAc$_2$ and Dol-PP-GlcNAc$_2$Man$_{1-2}$ at 37°, respectively),were screened for the activity of the GlcNAc-P transferase. The activity of this transferase in membrane preparations from alg1 and alg2 mutants is 18% and 55% of the wild type, respectively, at the non-permissive temperature,37°as compared to 60% and 90% for the mutant phenotypes at the permissive temperature,26°. The levels of mRNA for the GlcNAc-P transferase were estimated in these and other glycosylation mutants by the Northern Blot Method, using the transferase gene as a probe,(ALG7 in pJR41, Rine,J. et al.(1983) PNAS 80,6750).Two restriction fragments from pJR41,containing ALG7 sequences, were used as specific probes for the ALG7 mRNA levels. Our results indicate that in alg1 the levels of the ALG7 mRNA are significantly decreased. These and the enzymatic studies suggest that there is transcriptional and possibly metabolic regulation of the LLO pathway.

1532 MITOCHONDRIAL CYTOCHROME OXIDASE SUBUNIT II GENE IN THE YEAST HANSENULA SATURNUS. Janet Lawson and Donald W. Deters, Department of Microbiology, University of Texas, Austin, Texas. 78712-1095.

Mitochondrial biogenesis depends on the coordinated interaction of two physically separated genetic systems. A single 1.7 kb restriction fragment of mitochondrial DNA containing the cytochrome oxidase subunit II gene of the yeast Hansenula saturnus was identified by Southern hybridization using the subunit II gene of Saccharomyces cerevisiae as a probe. The fragment was isolated, cloned, and mapped. Smaller restriction fragments were subcloned into M13 derivatives and sequenced. The gene codes for a protein 247 amino acids long having a molecular weight of 28,000. H. saturnus mitochondria do not use the standard genetic code. As in fungal and mammalian mitochondria, TGA is a codon for tryptophan. H. saturnus mitochondria, in contrast to S. cerevisiae mitochondria, use CTT to code for leucine, not threonine. 22 codons are not used in the gene. Despite this extreme codon bias, 9 codons used to decode the sequence are not used to decode the equivalent gene in S. cerevisiae. Although the translated sequence is 80% homologous to that of S. cerevisiae, there are notable differences at the nucleotide level in the gene itself and in the flanking regions. The N-terminus of the translated gene, while significantly different from the S. cerevisiae sequence, is remarkably homologous to the corresponding sequence from the filamentous fungus Neurospora crasssa. An extraordinary number of inverted and direct repeats are found within the coding region of the gene. Several of these elements are conserved in subunit II genes of related mitochondria. Supported in part by grant F925 from The Robert A. Welch Foundation.

1533 MECHANISMS AND MACHINERY FOR PROTEIN IMPORT DURING MITOCHONDRIAL BIOGENESIS. Linda Marshall-Carlson, Jerry L. Allen and Michael G. Douglas, Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78284.

Using a combination of genetics and biochemistry, we are investigating import and assembly of nuclear-encoded mitochondrial proteins. Using the yeast F_1-ATPase β subunit as our model, a family of plasmids was constructed bearing various 5' portions of this gene fused to the E. coli lacZ gene. β-galactosidase activity was monitored to determine subcellular targeting of the hybrid protein in host cells. Subcellular fractionation, immunofluorescence and immunoelectron microscopy reveal that chimeric protein targeting and import appear to be a function of specific polypeptide regions of F_1-β. Only those fusions containing over 112 amino terminal residues were delivered to the mitochondrion and those containing over 273 amino acids disrupted normal function. Specific deletions have been made with the ATP2 portion of these hybrids and analysis is underway to further define residues crucial for import.

1534 Uptake of Proteins into Mitochondria: Analysis of ATP2-lacZ Fusion Products. M.T. McCammon[1], G. Garrett[2], S.D. Emr[2] and M.G. Douglas[1]. [1]Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas; [2]Division of Biology, California Institute of Technology, Pasadena, California.

A gene fusion study has been initiated to determine the protein signals and cellular machinery which participate in delivery of proteins to their functional locations within the mitochondrion. For this analysis, the structural gene coding for the yeast mitochondrial F_1-ATPase subunit (ATP2) was fused to the E. coli lacZ gene. The ATP2-lacZ gene fusions encode a family of hybrid proteins containing 9 to 350 amino terminal residues of the F_1- polypeptide (479 amino acids), fused to an enzymatically active portion of -galactosidase. Three classes of -Z hybrids were expressed in yeast: class I Z hybrids (less than 112 amino acids from F_1-) were localized to the cytoplasm, class II hybrids (between 139 and 210 amino acids of F_1-) were localized to mitochondria; class III hybrids (between 237 and 350 amino acids of F_1-) were also localized to mitochondria, but in such a way as to interfere with normal mitochondrial function. Second site conditional nuclear mutations have been isolated which restored mitochondrial function (see Garrett, McCammon, Douglas and Emr, abstract submitted).

1535 SECRETION OF MATURE MOUSE INTERLEUKIN-2 AND INTERLEUKIN-3 USING THE A-FACTOR SECRETORY SYSTEM, Atsushi Miyajima, Keiko Otsu, Craig Smith, Martha Bond, Donna Rennick, Naoko Arai, and Ken-ichi Arai. DNAX Research Institute, 1450 Page Mill Road, Palo Alto, California 94304.
By using the α-factor promoter and leader sequence, we have developed a general expression vector that permits secretion of mature proteins in S. cerevisiae. The coding sequences of the mouse T cell lymphokines, IL-2 and IL-3 (Yokota et al [1984] PNAS 81: 1070-1074 and PNAS in press), were fused separately to the first processing site of the α-factor precursor, which can be recognized by the KEX2 product. MATα cells carrying the hybrid genes expressed and secreted processed mouse IL-2 and IL-3 into the extra cellular space and culture media. The yeast-produced lymphokines had the biological activities expected. Yeast-IL-2 supported the proliferation of cloned mouse T cells. Yeast-IL-3 showed murine multilineage colony stimulating factor activity and promoted the growth and differentiation of hematopoietic cell types including multipotential stem cells and various committed progenitor cells.

1536 Endocytosis in Saccharomyces cerevisiae, Marja Pesonen, Recombinant DNA-Laboratory, University of Helsinki, Helsinki, Finland.

Upon incubation at 37°C with S. cerevisiae, two enveloped viruses, Vesicular stomatitis virus and Semliki Forest virus, became cell-associated. The association curve was linear for at least two hours. The cell-associated viruses were protected against protease digestions and mild detergent treatments. When applied to virions, the protease digestions degrade the envelope glycoproteins and the detergent releases the nucleocapsids. Thus, it appears, that the viruses were internalized. The uptake was concentration- and temperature-dependent. A soluble enzyme marker was taken up by S. cerevisiae likewise, the uptake displaying similar characteristics as that of the viruses. Novel homogenization and organelle fractionation techniques were applied to S. cerevisiae. The homogenization procedure resulted in nearly complete disintegration of the cells. At the same time, at most 10% of two vacuolar enzyme markers were found in a free form, indicating good preservation of at least the vacuoles during homogenization. The organelle fractionation procedure resulted in good separation with 80-90% recoveries of the vacuolar enzymes, plasma membrane markers, cell wall fragments and virions. Using these techniques the viral components were mainly found, after internalization, in a compartment which in organelle fractionation coincided partly with the plasma membrane markers and partly with none of the compartments mentioned above. A minor fraction coincided with the vacuole markers. This system will be used to trace the pathways of membrane traffic starting at the plasma membrane of the yeast cell.

1537 HETEROLOGOUS EXPRESSION OF A NATURALLY SECRETED PROTEIN MAY REQUIRE PROCESSING THROUGH THE YEAST SECRETORY PATHWAY FOR THE SYNTHESIS OF AN ACTIVE PRODUCT, D. Rogers, R. Kriz, K. Normington, K. Turner, Genetics Institute, Inc. 225 Longwood Avenue, Boston, Massachusetts 02115

The yeast glyceraldehyde-3-phosphate dehydrogenase promoter has been used to express high levels of heterologous protein. Cytoplasmic expression using this promoter yields 1-10% of total cellular protein as the expressed product. However, naturally secreted proteins are generally found to be inactive as cytoplasmic products. Disulphide bonds probably form during the secretion process and it is unlikely that they can form spontaneously in the cytoplasm. Correct folding and disulphide bridges are usually required for the biological activity of a naturally secreted protein. The genes for naturally secreted proteins were expressed to produce both cytoplasmic and secreted products. Total expressed protein and biologically active protein were determined in both the cytoplasmic and periplasmic fractions. Heterologous proteins which were found to be inactive as cytoplasmic expression products produced biologically active secreted products.

1538 MUTANTS DEFECTIVE IN VACUOLE PROTEIN SORTING, Joel H. Rothman and Tom H. Stevens, University of Oregon, Eugene, OR 97403.

During their transit through the ER and Golgi, newly synthesized secretory and vacuolar proteins use the early stages of the yeast secretory pathway. Since sec mutants defective in the late stages of the pathway block secretory but not vacuolar protein delivery, an early-acting sorting system must exist to segregate these proteins. To identify the genes whose products act to sort vacuolar proteins from other polypeptides we have devised a selection scheme allowing isolation of sorting mutants.

Using this selection we have identified a minimum of nine distinct complementation groups which when mutant result in mislocalization of the vacuolar zymogen CPY to the periplasm. A large number of these vacuole protein localization, or vpl, mutants are temperature-sensitive for growth and exhibit more pronounced CPY secretion at the non-permissive temperature. Many of these mutants act pleiotropically; in addition to CPY, other vacuolar proteins are also improperly sorted. This observation supports the hypothesis that a single intracellular sorting system operates on these proteins.

A negative selection scheme has been devised to allow rapid cloning of VPL genes and to isolate extragenic suppressor mutations. Using VPL clones we have isolated, we are determining whether VPL genes are essential for cell viability, and are constructing fusion proteins to examine the cellular location and function of their gene products. Results of gene disruptions and analysis of fusion proteins will be presented.

1539 YEAST MUTANTS THAT HAVE IMPROVED SECRETION EFFICIENCY FOR CALF PROCHYMOSIN, Robert A. Smith and Tina Gill, Collaborative Research, Inc., Lexington, MA.

When the gene for calf prochymosin is fused to the signal sequence from the yeast invertase gene and expressed in Saccharomyces cerevisiae only 1-2% is secreted into the medium. In an attempt to rectify this situation we have used a semi-quantitative screening assay to isolate EMS induced host mutations that secrete increased amounts of active chymosin. To date, approximately forty mutant strains have been isolated that secrete from two to five times as much active chymosin as the strain from which they were derived. In all cases the absolute expression level of prochymosin remains unchanged, only the distribution of prochymosin between the vacuole and the medium is affected. Complementation analysis indicates that mutations in at least four genes can give rise to this supersecreting phenotype. Interestingly the mutations in two of these complementation groups appear to cause a pleotropic sporulation defect, providing evidence for linkage between the sporulation and secretion pathways. We have been able to combine various mutations from different complementation groups into individual yeast strains and have found that in many cases the effects of the mutations are additive. Multiply mutant strains are now available that secrete up to 30% of the prochymosin they express.

1540 A LINEAR DNA PLASMID FROM KLUYVEROMYCES LACTIS WHICH ENCODES A SECRETED PROTEIN TOXIN. Michael J R Stark, Alan J Mileham, Michael A Romanos and Alan Boyd, The Leicester Biocentre, Leicester LE1 7RH, England.

In killer strains of the yeast Kluyveromyces lactis, export of a protein toxin is associated with the presence of two linear DNA plasmids, k1 (8.9kb) and k2 (13.4kb). The secreted toxin consists of three subunits (mol wts 95,000, 27,000 and 25,000 daltons) and genetic evidence suggests that the smaller plasmid encodes both the toxin and an immunity factor which protects the host cell. We have determined the nucleotide sequence of k1. Over 95% of the sequence is contained within 4 open reading frames (ORFs) of 995(ORF1),1146(ORF2),428(ORF3) and 249(ORF4) amino acids. Transcripts corresponding to ORFs2,3 and 4 in size, polarity and map position have been identified in K.lactis by Northern hybridisation, while primer extension experiments have shown that the 5'-nontranslated regions are particularly short (10-20 bases). A 13-base sequence conserved upstream from ORFs1,2 and 3 may play a role in their expression. Both ORFs 2 and 4 encode proteins with good potential N-terminal signal peptides,implying that they may be secreted. We have tentatively identified ORF2 as a toxin structural gene, since a deletion which lies entirely within this ORF abolishes toxin production. The C-terminal one-third of the putative toxin gene product contains three potential membrane-spanning hydrophobic segments and all 7 possible sites for N-linked glycosylation. This organisation of the ORF2 poly peptide suggests that the secreted toxin might be cleaved from a larger,glycosylated precursor, a model consistent with the fact that the coding capacity of ORF2 is well in excess of the size of even the largest toxin subunit. Experiments which will demonstrate the functions of the 4 gene products and their relationship to the 3 toxin subunits are in progress.

1541 DIFFERENTIAL EXPRESSION OF TWO NUCLEAR GENES FOR YEAST CYTOCHROME c OXIDASE SUBUNIT V, A MITOCHONDRIAL MEMBRANE PROTEIN. C.E. Trueblood, M.G. Cumsky, R.O. Poyton, University of Colorado, Boulder, CO 80309

In Saccharomyces cerevisiae, cytochrome c oxidase is composed of nine non-identical subunits: subunits I, I, and III are encoded in the mitochondrion while subunits IV, V, VI, VII, VIIa, and VIII are encoded in the nucleus. We have cloned two genes (COX5a and COX5b) that encode distinct forms of subunit V (Va and Vb). Strains with COX5a and/or COX5b gene disruptions were constructed and tested for cytochrome c oxidase activity. Strains with gene disruptions in COX5a and COX5b exhibit <1% cytochrome c oxidase activity, indicating that subunit V is required for assembly or catalytic activity. Disruption of the COX5a gene alone reduces the cytochrome c oxidase activity to 10% of wild-type levels, whereas disruption of COX5b does not measurably reduce cytochrome c oxidase activity. COX5a, therefore, contributes most or all of the subunit V in cytochrome c oxidase under the conditions of these experiments. When present on a high copy plasmid, the COX5b gene is capable of restoring nearly wild-type levels of respiration to strains deleted for COX5a, indicating that the Vb protein can function adequately. Experiments are underway to determine whether the differential expression of these two genes is affected at the level of transcription, RNA processing, translation, targeting, or assembly.

1542 STRUCTURAL DETERMINANTS THAT DIRECT PROTEINS TO THE VACUOLE, Luis Valls, Jr. and Tom H. Stevens, University of Oregon, Eugene, OR 97403.

Many hydrolases which are found in the yeast vacuole have been shown to be synthesized as inactive precursors having N-terminal extensions of 8-10 Kd, termed pro regions. PRC1 is the gene coding for one such protein, carboxypeptidase Y (CPY), and we have recently sequenced it. This is the first complete amino acid sequence deduced for a vacuolar or lysosomal protein, and reveals that CPY is synthesized as a 531 amino acid precursor, 111 amino acids larger than mature CPY. In addition, the first amino acids define a hydrophobic leader sequence. Amino-terminal sequencing studies are now under way, to determine whether this leader is cleaved upon translocation.

We are testing the hypothesis that the pro region of CPY serves at least two functions: to keep the protein inactive prior to vacuolar delivery and to provide it with a sorting determinant, or "address", that is recognized by the cellular sorting machinery. Our apprach is to mutagenize the PRC1 gene in vitro using hydroxylamine and in-frame deletion mutagenesis in the region which codes for pro to produce mutant forms of CPY which become mislocalized. Mapping and sequencing such mutations should precisely define the region of proCPY responsible for localization to the vacuole. Results of these studies will be presented.

1543 DELETIONS IN THE PRESEQUENCE OF THE YEAST F_1-ATPASE β-SUBUNIT AND MITOCHONDRIAL IMPORT. Alessio Vassarotti and Michael G. Douglas, Dept. of Biochemistry, Univ. of Texas Health Science Center, San Antonio, TX 78284.

A characteristic feature of most proteins delivered to mitochondria is the presence of a transient presequence at the amino terminal end. Using the mitochondrial F_1-ATPase β-subunit as a model, we have constructed a host-vehicle system to examine the role of its signal sequence in delivery and localization into mitochondria. We have utilized a PvuII restriction site located in the third codon of the ATP2 gene to construct a set of Bal31 generated deletions downstream from this site. High level expression of the in frame deletions was measured by expression of lacZ fused in frame at a downstream site. Analysis of expression of the ATP2-lacZ hybrid proteins with deletions ranging from 15 to 45 base pairs (i.e., the entire signal sequence) revealed that sufficient gene fusion product is delivered to mitochondria to inhibit the development of functional organelles. This observation would suggest that the F_1-β presequence is not essential for its in vivo mitochondrial delivery. In order to further examine the role of the signal sequence, we have reconstructed an otherwise wild-type ATP2 gene containing the presequence deletions. Analysis of expression of these constructions in an ATP2 deletion host strain is currently in progress.

1544 STRUCTURE AND SURFACE LOCALIZATION OF A YEAST AGGLUTININ. Keith G. Weinstock and Clinton E. Ballou, University of California, Berkeley, CA 94720.

Saccharomyces kluyveri is a heterothallic yeast that exhibits a strong, constituitive, sexual agglutination when cells of opposite mating type (termed 16- and 17-cells in this species) are mixed. Two glycoproteins mediate the cell adhesion and can be solubilized from the cell wall using beta-1,3-glucanases. The 16-agglutinin is a large heat-stable glycoprotein that contains a single recognition site which is released by treatment with disulfide reducing agents. The activity of the 17-agglutinin is heat-labile but is unaffected by disulfide reducing agents. Solubilization of the cell wall from 17-cells with Zymolyase (a crude beta-1,3-glucanase), followed by affinity purification, yielded a glycoprotein of 60 kilodaltons (K). A second species of 134K was subsequently purified. Treatment of this larger protein with Zymolyase rapidly produced the 60K protein. Antibody prepared against the 134K and 60K proteins specifically precipitated a heterogeneous, high molecular weight protein (>200K) from material solubilized by digestion of isolated cell walls with a purified beta-1,3-glucanase. Immunofluorescence of whole 17-cells shows general surface staining with, sometimes, pronounced staining in the regions of the bud scars and at the sites of nascent bud formation.

1545 GENETIC ANALYSIS OF SIGNAL RECOGNITION PARTICLE FUNCTION, Jo Ann Wise, University of Illinois, Urbana, IL 61801

Small RNAs (≤300 nucleotides), complexed with protein, have recently been discovered to participate in an unexpectedly diverse array of cellular processes, including transcriptional control, translational control, messenger RNA splicing, tRNA 5' trimming, and protein secretion. My laboratory is currently focusing its attention on 7SL RNA, identified by Walter and Blobel as a component of Signal Recognition Particle (SRP). They showed by in vitro reconstitution experiments with a mammalian system that SRP, which contains six non-identical polypeptides in addition to the RNA, plays a role in targeting pre-secretory proteins to the endoplasmic reticulum (ER) membrane. To examine in vivo the mechanism by which SRP recognizes ribosomes translating messages encoding proteins with signal sequences, we will employ genetic strategies uniquely available in yeast. The effects of mutating SRP components will be assessed in cells transformed with genes altered in vitro. As an initial step, we have identified three recombinant clones which encode yeast RNAs simi-lar in size to 7SL from higher eukaryotes; further evidence for equivalence is being sought through structural analysis and transcription studies. Once a positive correlation has been made, mutagenesis will proceed. The consequences of a null allele will indicate whether 7SL RNA is essential in yeast, and analysis of point mutations should reveal features of the RNA which are indispensable for function. By selecting for second-site suppressors of secretory defects resulting from mutations in 7SL, we should be able to identify SRP proteins as well as other components of the export machinery.

1546 PRE-PRO-α-FACTOR DIRECTED SECRETION OF HUMAN PROTEINS FROM SACCHAROMYCES CEREVISIAE. K. M. Zsebo, Edward M. Lau, Sidney V. Suggs and Grant A. Bitter, Amgen, 1900 Oak Terrace Lane, Thousand Oaks, CA 91320

Fusions between the MFα structural gene and chemically synthesized genes encoding human proteins have been constructed. The gene fusions encode hybrid proteins which include the pre-pro-α-factor leader region fused to the human protein. A spacer peptide which includes proteolytic recognition sequences involved in maturation of the α-factor peptides from the native precursor immediately precedes the foreign peptide in the hybrid precursor. These fusions direct secretion of both small (ß-endorphin, calcitonin; 31 amino acids) and large (consensus α-interferon, 166 amino acids) proteins into the culture medium. In stationary phase cultures, 99% of the synthesized ß-endorphin and calcitonin is secreted into the culture medium. In contrast, 90% of the synthesized consensus α-interferon (IFN-αCon_1) remains associated with the cell wall.

These secreted proteins contain NH_2 terminal Glu-Ala dipeptides due to incomplete processing by dipeptidyl amino peptidase A. Site-directed mutagenesis was utilized to generate a cloning vector which allows assembly of gene fusions containing any desired spacer peptide. An MFα/IFN-αCon_1 gene fusion was constructed which included the trypsin-like cleavage sequence but deleted the Glu-Ala codons. This gene fusion programmed secretion of IFN-αCon_1 with accurately processed NH_2 terminii. The effects of mutations in the KEX2 gene on efficiency of secretion as well as previously observed internal cleavages (Bitter, et al., PNAS 81, 53350-5334) is documented.

1547 Mitochondrial Topogenic Sequences. Jim Kaput, Sherry Goltz, Marjorie Brandriss*, Carl Peter Blobel, and Gunter Blobel. The Rockefeller University, New York, NY. and *UNJMDS, Newark, NJ.

Eukaryotic cells have evolved complex intracellular organelles that effectively separate biochemical pathways. However, most of the proteins involved in the reactions must be imported into the organelles across one or more membranes. A striking example of this transport process occurs with mitochondria, which not only imports over 90% of its proteins from the cytoplasm, but also segregates the proteins to the outer membrane, the intermembrane space, the inner membrane, or the matrix. The information required for translocation and segregation is presumably encoded in discrete segments (topogenic sequences) of the polypeptide chain (Blobel [1980] PNAS 77, 1496-1500). The presence, location, and function of the putative topogenic sequences in Δ^1-pyrroline-5-carboxylate (P5C)-dehydrogenase (a matrix-localized protein) and cytochrome c peroxidase (CCP; an intermembrane space protein) are being analyzed by reverse genetic techniques. Fusion proteins consisting of the NH_2-terminus of mitochondrial precursor proteins and nonmitochondrial proteins have been constructed and analyzed in an in vitro transcription/translation/translocation system that greatly speeds the construction and analyses of mutated gene products. These fusion proteins are defining the protein segment that may contain the putative mitochondrial topogenic sequence. The exact protein segment that constitutes the topogenic sequence is being defined through computer analyses of mitochondrial precursors and through analyses of site-specific mutations.

1548 Nuclear and mitochondrial tRNA methyltransferases appear to be coded by the same nuclear gene in Saccharomyces cerevisiae. [2]Nancy C. Martin,[1] Steven R. Ellis,[1] Michael J. Morales,[1] and Anita K. Hopper[2]. University of Texas Health Science Center, Dallas, Tx., Hershey Medical Center, Hershey, PA.

Nuclear mutations at the trm1 and trm2 loci in S. cerevisiae abolish the synthesis of m^2_2G and m^5U respectively in nuclear and mitochondrial tRNAs. The enzymes which carry out these modifications cannot be detected in in vitro assays using whole cell or mitochondrial extracts of the mutants but are readily measured in comparable wild type preparations. Since both m^2_2G and m^5U are present on yeast nuclear precursor tRNAs, we infer that the methyltransferases are located in the nucleus. A nuclear location of the m^5U methyltransferase has been demonstrated by injection experiments using Xenopus oocytes.

A 2500 base pair wild type yeast genomic fragment that corresponds to the TRM1 locus has been obtained. Northern analysis shows that a 1700 base poly A+RNA is derived from this fragment. Mutant cells transformed with plasmids carrying this cloned fragment have m^2_2G in both nuclear and mitochondrial tRNA. The m^2_2G dimethyltransferase activity is present in such transformants and is elevated with respect to wild type, when a multicopy vector is used suggesting that TRM1 codes for m^2_2G dimethyltransferase. To determine if different forms of a methyltransferase are encoded by the same gene or whether identical proteins might be found in both the nucleus and mitochondria we are sequencing the DNA and analyzing the protein products of the TRM1 gene. (Supported by NIH GM27597).

1549 IMPORT OF PROTEINS INTO MITOCHONDRIA Richard Zimmermann
Institut für Physiologische Chemie, D 8000 München 2, Federal Republic of Germany

Data will be presented regarding the following central aspects in the import of proteins into mitochondria:

I,Receptors on the Mitochondrial Surface

Binding studies using purified precursor proteins of cytochrome c and porin.

II,Membrane Transfer in Import of Mitochondrial Proteins

Novel intermediates of the ADP/ATP carrier and the ß subunit of F_1ATPase.

III,Membrane Potential

IV,Processing Peptidase(s)

Reference:R.Zimmermann,Import of Proteins into Mitochondria in "Protein Compartmentalization" (Boime,I. et al., eds) Springer Verlag, New York.