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FACTORS AFFECTING HOMOLOGOUS AND HETEROLOGOUS GENE EXPRESSION IN *SACCHAROMYCES CEREVISIAE*

J. MELLOR, M. J. DOBSON, M. F. TUITE, N. A. ROBERTS, A. J. KINGSMAN AND S. M. KINGSMAN

Department of Biochemistry, South Parks Road, Oxford OX1 3QU

We have used the expression signals from the yeast *PGK* gene and the replication origin from the 2μ circle to construct high efficiency expression vectors for use in yeast. These vectors direct the synthesis of homologous and heterologous polypeptides in yeast. The coding sequences for human interferon-alpha 2 and yeast *PGK* were placed under the control of the *PGK* 5′ region and the steady state levels of the two polypeptides, expressed from these plasmids, were compared. The levels of *PGK* were about 50-fold higher than the levels of interferon. We have started an analysis of this marked difference in the efficiencies of heterologous and homologous gene expression in yeast.

We have shown that although the interferon polypeptide is turned over more rapidly than *PGK* this is insufficient to account for the large difference in steady state levels; a 10-fold difference remains even after a short pulse label. These data suggest that a major component in the differences in steady state protein levels reflect differences in the rate of protein synthesis. The limiting step may be at the RNA level or the efficiency of translation. It is unlikely that the rate of protein synthesis is affected at translation initiation, as the nucleotide sequences of the initiation environment in one of our interferon constructions are identical to the *PGK* construction. The interferon coding sequence contains many codons which are used rarely by yeast genes and therefore the rate of translation elongation may be limited by the availability of charged tRNA species. In a Northern blot analysis, however, we have shown that the steady state levels of *PGK* RNA are 10-fold higher than interferon RNA levels. This result was surprising, as the promoter regions used in these constructions are identical. These data indicate that the expression of heterologous genes in yeast may be limited primarily at the RNA level. This may be due either to inefficient transcription or enhanced lability of heterologous RNA. The resolution of these alternatives will be discussed.

IDENTIFICATION AND CHARACTERISATION OF FUNCTIONAL REGIONS WITHIN THE YEAST TRANSPOSON, Ty

A. M. FULTON, M. J. DOBSON, J. MELLOR, W. WILSON, B. A. BOWEN, M. F. TUITE, S. M. KINGSMAN AND A. J. KINGSMAN

Department of Biochemistry, South Parks Road, Oxford OX1 3QU

The yeast transposon, Ty, is present at about 30–35 copies per haploid genome. It is composed of a 5·0 kb epsilon region flanked by directly repeated 350 bp delta sequences. The Ty family is heterogeneous but broadly resembles the structure of avian and mammalian retroviral proviruses. While it has been shown that Ty sequences affect the expression of neighbouring genes, almost nothing is known of the internal organisation of the elements. Two RNA species corresponding to a full length (5·6 kb) and a shorter (5·0 kb) transcript have been described.

We have identified functional open reading frames within the Ty element by making fusion proteins with *E. coli* B-galactosidase using a *lacZ* DNA fragment lacking the first 7 codons of the gene. These data suggest that Ty elements might encode protein products. The promoter activity of Ty elements is weak. In order to detect possible Ty encoded polypeptides, a promoterless Ty element was placed under the control of the efficient promoter from the

yeast *PGK* gene. When this construction was introduced into yeast on a high copy number plasmid, we detected two new basic polypeptides of molecular weight 47 K in yeast protein extracts. This is the first evidence that Ty elements encode proteins.

We have also used the B-galactosidase fusions to assay Ty promoter functions in a series of Ty deletion derivatives made *in vitro*, enabling us to map the location of a Ty promoter.

A VEHICLE FOR THE EFFICIENT EXPORT OF HETEROLOGOUS PROTEINS FROM YEAST: SYNTHESIS, PROCESSING, AND SECRETION OF THE YEAST MATING PHEROMONE *a*-FACTOR AND HYBRID PROTEINS DERIVED FROM IT

JEREMY THORNER, DAVID JULIUS, LINDLEY BLAIR, MONICA FLESSEL, DEBRA BARNES AND RIYO KUNISAWA

Department of Microbiology and Immunology, University of California, Berkeley, California, USA 94720

Cells of the yeast *Saccharomyces cerevisiae* contain two structural genes that encode a precursor to the secreted peptide mating pheromone, *a*-factor. One gene (*MFa*1) encodes a precursor, prepro-*a*-factor A, that contains four exact copies of the mature pheromone, *a*-factor 1. This gene is polymorphic in yeasts related to *S. cerevisiae*. In *S. carlsbergensis* and *S. diastaticus*, *MFa*1 encodes a precursor that contains only three pheromone units; in *S. oviformis*, *MFa*1 specifies a precursor that contains five pheromone repeats. The other gene (*MFa*2) encodes a precursor, prepro-*a*-factor B, that contains two copies of the mature pheromone. One pheromone is identical to the *a*-factor 1 made by prepro-*a*-factor A; the other pheromone is an apparent variant (*a*-factor 2) which differs from *a*-factor 1 by two amino acid substitutions of similar kind (Lys → Arg and Asn → Gln). As judged by the *in vitro* translation capacity of mRNA, electrophoretic analysis of mRNA, and gene disruption experiments, *MFa*1 is the major pheromone structural gene in that it is expressed at levels at least two orders of magnitude greater than *MFa*2. The translocation, glycosylation, and proteolytic processing of prepro-*a*-factor A have been studied in a series of mutants thought to block the general pathway for secretory transport in yeast (*sec* mutants). In addition, the nature of the biochemical lesions that prevent *a*-factor production in a variety of other *a* cell-specific non-mating ("sterile") mutants, including *ste*13 and *kex*2, have been defined. The *STE*13 and *KEX*2 genes have been isolated by recombinant DNA methodology and characterised. Based on our understanding of the mechanisms of *a*-factor synthesis, processing, and secretion, it has been possible to use recombinant DNA techniques to fuse the prepro-leader portion of prepro-*a*-factor A to the genes for a variety of mammalian peptide hormones and other secreted proteins, including epidermal growth factor, proinsulin, β-endorphin, rennin, and interferon. Remarkably, for most of these constructions, the mammalian protein is properly processed and efficiently secreted from yeast cells.

RECOMBINATIONAL "HOT SPOTS" IN *SACCHAROMYCES CEREVISIAE*

JAMES E. HABER

Brandeis University, Waltham, MA 02254, USA

We have been interested in the ways in which both mitotic and meiotic recombination are initiated.

Mechanism of homothallic switching of yeast mating type genes

Switching of the yeast mating type (*MAT*) genes may be viewed as a specialised form of repair of a double-strand chromosome break, resulting in a intrachromosomal mitotic gene conversion event. Efficient switching requires that the donor and recipient loci be on the same chromosome, but not necessarily in the same orientation. Normal donor preferences can be changed by moving another donor closer to *MAT*. Switching is apparently initiated by a double-strand break within the *MAT* locus, at a site near the Y/Z border; mutations in this region prevent switching. Despite the fact that these *MAT-inc* mutations are not substrates for initiating switching, they can be transposed efficiently to other *MAT* loci. Deletions

removing as much as 100 bp from the Y/Z border can nevertheless be transposed efficiently from *HMR* to *MAT*. Thus, if a double-strand break in the *MAT* Y/Z region initiates switching, it is possible to complete the event even if sequences homologous to both ends of the double strand-break are missing. Various types of mutations preventing switching have been isolated; the specific steps blocked in *inc*, *stk*1, *HO*-1, and *swi*1-1 will be discussed.

The Y/Z region can also be moved to other chromosomal locations as a portable region of site-specific mitotic recombination, regulated by *HO*. Results of studies where the Y/Z region has been inserted between a tandem duplication of *URA*3 genes on chromosome V will be discussed.

Analysis of two regions with high frequencies of meiotic recombination in yeast

We have focused our attention on two adjacent regions in a yeast chromosome, to determine: a) when meiotic recombination occurs, relative to the times of DNA synthesis and commitment to recombination, and b) if there are "hot spots" for the initiation of crossing over.

A 9 kb region containing a duplication of *MAT* genes separated by *URA*3 and pBR322 exhibits crossing over in more than 15 per cent of the tetrads. Because *MATa* contains a *Bg*1II site not found in *MATα* or in the plasmid sequences, recombinants can be readily detected on Southern blots. This makes it possible to monitor directly the timing of meiotic recombination. We have been able to detect the appearance of recombined DNA approximately 6 hours after meiosis is initiated, just after the increase in pre-meiotic DNA synthesis and coincident with the time at which prototrophs can be detected by returning cells to selective growth medium. This system should make it possible to examine the effect of various meiotic-deficient mutations on the actual formation of recombined DNA.

By introducing various deletions and genetic markers into this region we can subdivide it into smaller intervals to determine where exchanges have actually occurred and whether there is a specific "hot spot" for meiotic crossing over. By and large, recombination in sub-regions of the interval was proportional to physical length. Thus, despite the fact that this interval exhibits higher than average levels of meiotic recombination, no one segment contains a unique meiotic hot spot.

In contrast, the adjacent 21 kb interval between *cry*1 and *MAT* exhibits a very different pattern, with nearly all recombination occurring in the distal half of the interval. The 10 kb region near *cry*1 is remarkably "cold", with only 1 exchange in more than 230 tetrads. The effects of a dominant enhancer of recombination specific to this region will also be discussed.

IDENTIFICATION AND CHARACTERIZATION OF YEAST DNA TOPOISOMERASE MUTANTS

ROLF STERNGLANZ, STEPHEN DINARDO, KAREN VOELKEL, CATHERINE THRASH
Department of Biochemistry, State University of New York, Stony Brook, NY 11794, USA

A collection of temperature-sensitive mutants of the yeast, *S. cerevisiae*, has been screened for DNA topoisomerase defects. A temperature-sensitive DNA topoisomerase II mutant has been identified. Genetic analysis shows that a single recessive nuclear mutation is responsible for both temperature-sensitive growth and enzymatic activity. Thus topoisomerase II is essential for viability and the mutation is most probably in the structural gene. Experiments with synchronised cells show that at the non-permissive temperature cells can undergo one, and only one, round of DNA replication. Microscopic examination shows that these cells are arrested at medial nuclear division. Analysis of 2 micron plasmid DNA from these cells shows it to be in the form of multiply intertwined catenated dimers. The results suggest that DNA topoisomerase II is necessary for the segregation of chromosomes at the termination of DNA replication. Evidence that topoisomerase II may also have another role in the yeast cell cycle will also be presented.

A DNA topoisomerase I mutant has also been identified. The mutant has normal topoisomerase I activity when assayed at 25° and about 20 per cent of normal activity when assayed at 36°. The mutation causing the topoisomerase I defect can be separated from the mutation causing temperature-sensitive growth. Strains with only the topoisomerase I mutation grow normally at all temperatures tested. The mutation has been mapped to the *MAK*1 gene. Three previously isolated *mak*1 mutants exhibit less than 1 per cent of normal topoisomerase I activity in our assay, but yet they grow normally. The implications of these results for the role of DNA topoisomerase I in the cell will be discussed.

CONTROL OF EXPRESSION OF DNA LIGASE IN YEAST

L. H. JOHNSTON AND D. G. BARKER

Microbiology Division, National Institute for Medical Research, Mill Hill, London

The development of a novel assay for DNA ligase has enabled us to quantitate levels of the enzymes in crude extracts. This assay is sufficiently sensitive to detect the low levels of ligase remaining in the conditional *S. cerevisiae* mutant *cdc*9 and to show that this activity is temperature-sensitive, thus confirming that *cdc*9 is a structural gene for DNA ligase. The assay has also enabled us to show that ligase is induced some 3–5 fold by UV-irradiation and preliminary data suggests it is also induced by heat shock. Although we have yet to determine whether it fluctuates in the cell cycle, it is already clear that control of ligase expression is likely to be interesting. To analyse this control at the molecular level we have cloned the *cdc*9 gene and also the *cdc*17 gene from the fission yeast *Schizosaccharomyces pombe*, which also encodes a DNA ligase. By use of a plasmic vector able to replicate in both yeasts we have found that the *cdc*9 gene from budding yeast is able to complement the *cdc*17 mutation in *S. pombe*, but the reverse complementation does not work. We have now sequenced both genes and a brief comparison will be presented.

CELL CYCLE CONTROL IN THE FISSION YEAST

PAUL NURSE

School of Biological Sciences, University of Sussex, Falmer, Brighton

There are two major points for cell cycle control in fission yeast. One acts in late G1 before *S*-phase and the other in G2 before mitosis. One gene *cdc*2 functions at both control points. This gene and an extragenic suppressor of certain *cdc*2 mutants called *suc*1 have been cloned and the cloned genes used to identify the *cdc*1 and *suc*1 transcripts. The characterisation and regulation of these transcripts will be described.

FUNCTIONAL AND STRUCTURAL ORGANISATION OF rDNA IN *SACCHAROMYCES CEREVISIAE*

DONALD WILLIAMSON AND SHIRLEY McCREADY

National Institute for Medical Research, Mill Hill and Botany School, University of Oxford

Ribosomal RNAs in brewer's yeast are coded by a 9 kb sequence of DNA which is reiterated about 120-fold in the haploid genome. It is currently believed that all the rDNA repeats (each of which includes about 2 kb of so-called "non transcribed" spacer) are identical, and form a continuous head-to-tail array about 1000 Kb long, on chromosome XII. The mechanics of replication of this array are not clear, and neither the distribution of replicons along the array, nor the locations of replication origins within the repeat units, are known.

Recently, sedimentation analysis of pulse-labelled rDNA led Walmsley *et al.*, (Cold Spring Harbor meeting on *The Molecular Biology of Yeast*, 1983, p. 68) to conclude that rDNA replicons are about 5 repeat units in length, and only one in five of the repeats may function as a replication origin. This means that if the repeats are identical, determination of the sites of initiation must involve factors other than simply the primary sequence. However, restriction analysis has now shown us that high molecular weight rDNA molecules are cut infrequently by various restriction enzymes which have not hitherto been shown to cut isolated repeat units. This might be explained either by a low level of restriction site heterogeneity of the repeat units or by the organisation of the rDNA into clusters separated by non-rDNA spacers. The implications of these observations for the siting of replication origins in the array will be discussed.

YEAST TELOMERES AND ARTIFICIAL CHROMOSOMES

J. W. SZOSTAK

Dana-Farber Cancer Institute, and Department of Biological Chemistry, Harvard Medical School, Boston MA, USA 02115

Telomeres are special DNA structures that provide stable, fully replicatable DNA termini. We have used the ends of the linear extrachromosomal rDNA of *Tetrahymena* to study the

structure and function of telomeres in yeast. The *Tetrahymena* ends work as stable ends on linear plasmids in yeast. Deletion mapping experiments have shown that the only sequences required for this function are the cluster of C–C–C–C–A–A repeats found next to the terminus of the rDNA. We have cloned yeast telomeres by removing one *Tetrahymena* end from a linear plasmid by restriction enzyme digestion; such DNA transforms yeast very poorly, and thus provides a powerful selection for the presence of a functional telomere. Yeast telomeres are similar in many ways to the *Tetrahymena* end, but have a different sequence composed of irregular repeats: C_{1-3}–A. *Tetrahymena* ends become larger during replication in yeast by about 200 bp. This increase in size is due to the addition of sequences similar to those found in yeast telomeres. Possible mechanisms for this reaction will be described.

We have constructed artificial chromosomes by combining cloned origins of replication, centromeres and telomeres. Centromeres do not function properly on short linear plasmids (<15 kb), but do appear to work well on long linear plasmids (>50 kb). Such artificial chromosomes pair with each other and disjoin properly in meiosis. The fidelity of both mitotic and meiotic segregation is lower than that of natural chromosomes. Possible reasons for this, and for the effect of size, will be discussed. The telomeres and adjacent sequences of yeast chromosome III have been removed and substituted with *Tetrahymena* telomeres; the resulting chromosome appears to function normally in mitosis and meiosis.

TRANSFORMATION OF YEAST FROM ψ^- TO ψ^+

HWA DAI, PATRICIA LUND AND B. S. COX

Academica Sinica, Taipei, Taiwan and Botany School, South Parks Road, Oxford

ψ is a non-Mendelian determinant of *Saccharomyces cerevisiae* that affects the efficiency of certain tRNA-encoding translocational suppressors by controlling the presence of a ribosome-associated factor (Tuite *et al.*, *Proc. Natl. Acad. Sci. USA, 80,* 2824, 1983 and in press). The nature of the determinant is unknown. The genetic data are consistent with the presence of a cytoplasmically-located stable plasmid.

We show that ψ^- yeast can be transformed to ψ^+ by something that co-purifies with supercoiled DNA extracted from either [*cir*+] or apparently "[*cir*-9]" ψ^+ strains.

THE USE OF *CUP*1 AS A SELECTABLE MARKER IN YEAST TRANSFORMATIONS

R. C. A. HENDERSON, B. S. COX AND R. TUBB

Botany School, South Parks Road, Oxford and The Brewing Research Foundation, Nutfield, Surrey

At present, transformation of yeast depends upon selecting prototrophic cells from an auxotrophic recipient strain. Genetic modification of commercial strains, which are prototrophic, demands a different system. We have tested the use of the *CUP*1 gene as a selectable marker for the transformation of brewing yeasts and others. *CUP*1 has been cloned and extensively characterised by Fogel and Welch (*Proc. Natl. Acad. Sci. USA, 79,* 5342, 1982) and by us. Its most important properties are that it is a dominant gene conferring a level of copper resistance that correlates with its copy-number and that expression of its product, a copper-chelating protein, is inducible by low levels of copper.

We have compared the use of *CUP*1 with the *LEU*2 gene as a selectable marker in transformations of copper-sensitive, *leu*2 yeast. *CUP*1 can be utilised in a variety of selection protocols and, because of the stringency of selection can be varied easily, is more flexible in use than is prototrophy. Very high efficiency of transformation (up to 10^4 μg DNA) can be achieved.

We have successfully transformed six different brewing strains of yeast to copper resistance. We have shown that the copper resistance is plasmid borne in each case. It is clear that *CUP*1-based selection systems are feasible for genetic manipulation in prototrophic commercial yeasts.

THE ORGANISATION AND BEHAVIOUR OF Ty ELEMENTS IN YEAST

B. A. BOWEN, M. F. TUITE, A. M. FULTON, S. M. KINGSMAN AND A. J. KINGSMAN
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU

Signals for Ty transcription reside in the 5′ terminal delta sequence. The organisation of different 5′ deltas will be analysed in detail. Sequences leading out of the 5′ delta into the central region have been determined for two Ty elements. An open reading frame (ORF) extending for at least 60 codons is preserved in each. This ORF initiates in the 5′ delta and can be translated *in vivo*. The amino acid sequences encoded by each ORF differ mainly by a number of conservative changes.

Specific probes have been constructed for Ty 1–15 and Ty 1–17, which differ by two large substitutions. Sequences homologous to these probes are present at about 15–20 and 10 copies per cell, respectively. The larger substitution in Ty 1–17 was replaced with a gene conferring resistance to the antibiotic G418. Selection for high levels of G418 resistance in cells transformed with this molecule results in gross rearrangement of Ty sequences in the genome.

SYNTHESIS OF *ESCHERICHIA COLI* DNA POLYMERASE IN *SACCHAROMYCES CEREVISIAE*

S. G. SEDGWICK AND A. SPANOS
Genetics Division, National Institute for Medical Research, London NW7 1AA

E. coli DNA polymerase I is involved in DNA repair and has an ancillary role in chromosomal replication. The structural gene for this enzyme, *po*1A$^+$, has now been expressed in *S. cerevisiae* as a first step in a complementation analysis of repair and replication deficiencies in yeast. Plasmid shuttle vectors were used, which had a low copy number in *E. coli*, and which carried either *ars*1 or 2 μ replicative sequences. Synthesis of bacterial DNA polymerase in yeast was demonstrated by (i) the differential inhibition of DNA polymerase I and yeast polymerases by aphidicolin, N-ethyl maleimide, and p-chloromercurobenzoate, (ii) the molecular weights of polymerase activity assayed *in situ* after SDS-polyacrylamide gel electrophoresis, (iii) differences in substrate affinities. Introduction of *po*1A$^+$ plasmids into yeast raised polymerase levels approximately 5-fold under yeast DNA polymerase assay conditions. The *po*1A$^+$ gene was expressed in yeast without the aid of yeast promotor sequences. However, deletion of the cloned insert preceding bp-100 prevented expression in yeast, but not in *E. coli*, indicating that the two organisms use different sequences for *po*1A$^+$ expression. Cloning of *po*1A$^+$ into high bacterial copy number vectors by ourselves, and others, was uniformly unsuccessful. The use of low copy number shuttle vectors may therefore provide a means of cloning genes whose excessive expression is lethal in *E. coli*.

ENDOGENOUS NONSENSE SUPPRESSOR tRNAS IN EUKARYOTES: A tRNA FROM *CANDIDA ALBICANS* ABLE TO TRANSLATE BOTH UAG AND UGA CODONS *IN VITRO*

M. F. TUITE, P. A. BOWER AND C. S. McLAUGHLIN
Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ and Department of Biological Chemistry, University of California, Irvine CA 92717, USA

Increasing evidence from both prokaryotes and eukaryotes has suggested that the regulated readthrough of termination codons may define a novel mechanism for regulating gene expression at the level of translation. We have examined the ability of tRNAs, isolated from a number of different eukaryotes, to translate either the UAG or the UGA termination codons in a cell-free system from *Saccharomyces cerevisiae*.

This study identified a tRNA from the dimorphic fungus *Candida albicans* which not only efficiently translates *both* the UAG and UGA codon, but also causes some degree of mistranslation of natural mRNAs (but not poly(U)). The tRNA is found in both hyphal and yeast phase cells of *C. albicans* and, unlike *S. cerevisiae* nonsense suppressor tRNAs (Tuite *et al.*, *Proc. Nat. Acad. Sci. USA*, *80*, 2824, 1983), works efficiently in lysates prepared from both [*psi*$^+$] and [*psi*$^-$] strains of *S. cerevisiae*. Its *in vitro* effects are also observed in cell-free systems prepared from other eukaryotes.

The tRNA can be separated from other *C. albicans* tRNAs by two-dimensional electrophoresis, and preliminary evidence suggests that it can be charged with [^{3}H]-leucine.

Possible models to explain the *in vitro* behaviour of this apparently unique eukaryotic tRNA will be discussed.

ZYGOSACCHAROMYCES BAILII—A NEW HOST?

B. M. PEARSON, D. A. MACKENZIE, K. A. PAINTING AND M. H. J. KEENAN

Food Research Institute, Colney Lane, Norwich NR4 7UA

Small-scale preparative techniques, followed by 2D electrophoresis and electron microscopy have revealed the presence of two plasmids in strains of *Zygosaccharomyces bailii*, a common food spoilage yeast. These plasmids, designated pZB1 and pZB2, are about 6·0 kb and 5·4 kb long respectively. One isolate of *Z. bailii*, NCYC 1427 contains only one plasmid which is homologous to pZB2 but distinct from pZB1 and the 2 μm plasmids of *Saccharomyces cerevisiae* and *Z. rouxii*. Restriction analysis indicates that pZB2 has at least three unique sites. Investigations are under way to characterise the larger pZB1 plasmid.

The possibility of using these yeasts as recipients of recombinant DNA is being explored.

ANTISUPPRESSORS ACTING ON SUPPRESSOR *suaC*109 OF *ASPERGILLUS NIDULANS*

S. D. MARTINELLI

Department of Botany, Birkbeck College, University of London, Malet Street, London WC1E 7HX

*suaC*109 is an allele specific suppressor which acts on 10 alleles in eight different genes. It is a pleiotropic mutation conferring cold sensitivity, aminoglycoside sensitivity and slow growth on all strains containing this suppressor. All four characters segregate together in crosses. *suaC*109 is suspected to be a ribosomal mutant. After treatment of *suaC*109 by ultraviolet mutagenesis cold resistant revertants were isolated. In crosses to wildtype, about half those tested proved to be intragenic revertants. However, eight reverted to cold resistance at another locus. Of these, seven also had the phenotype of antisuppressors. This property segregated with cold resistance in crosses. In growth tests on the aminoglycoside antibiotic—hygromycin, the seven antisuppressors were shown to partially compensate the hypersensitive phenotype of *suaC*109. Thus it is possible to revert several facets of the suppressor's phenotype simultaneously.

In crosses between the antisuppressor mutants, designated *asu*-5, -11, -13, -14, -15, -16, and -26, at least five loci have been identified so far. The antisuppressors have also been crossed to other suppressors. Ribosomal-type suppressors with similar properties to *suaC*109, *i.e.*, *suaA*101 and *sua*-115 are generally affected by antisuppressors, whereas suppressors *suaD*108, *D*103 and *B*111, the tRNA like suppressors, have only been affected in one combination, *i.e.*, *suaD*103 and *asu*-13, so far.

The antisuppressor phenotype is most easily recognised in an *a*1*X*4 background on media containing allantoin as nitrogen source.

HYGROMYCIN RESISTANCE IN *ASPERGILLUS NIDULANS*

A. ZAMIR AND S. D. MARTINELLI

Department of Botany, Birkbeck College, University of London, Malet Street, London WC1E 7HX

We are interested in collecting many different types of ribosomal mutants. Hygromycin is an antibiotic known to affect ribosomal functions and it is inhibitory at low concentrations to *A. nidulans*. Therefore, we have isolated hygromycin resistant mutants. Resistant sectors are produced when sensitive colonies are grown on hygromycin. Sectors have been isolated from strain 390 (*fwA*1, *pabaA*1, *a*1*X*4, *sB*43) and strain 384 (as 390, plus suppressor *sua*-115). Resistant cultures have a higher radial growth rate on hygromycin than the original strains.

Resistant mutants have been crossed to strain 12 (*panto B*100) thought to represent a wildtype. However, it was discovered subsequently that strain 390 is more resistant to high concentrations of antibiotic than strain 12, thus complicating the cross analysis. In addition, it is very difficult to score progeny for hygromycin resistance on multiply inoculated replica-plates. Consequently, our argument is based mainly on qualitative assessment, with support from quantitative data.

Resistance mutations segregated in crosses between strain 12 and three derivatives of strain 390; a ratio of approximately 3 resistant: 1 sensitive colony was seen. Within the resistant class, progeny subdivided into 1 very resistant: 2 moderately resistant. Very resistant progeny were similar to the mutants, and moderately resistant to strain 390. We suggest that two different resistance genes are segregating and that the resistant sectors contain both resistant (mutant) alleles whereas strain 12 contains neither.

sua-115 confers hygromycin hypersensitivity on strain 384. Resistant isolates from strain 384 partially compensate this hypersensitivity. This partial resistance segregates in crosses, and is independent of *sua*-115 itself. The segregation of resistant alleles is being investigated in crosses homozygous for *sua*-115.

SOME NEW SUPER-SUPPRESSORS IN *ASPERGILLUS NIDULANS*

R. BRATT AND S. D. MARTINELLI

Department of Botany, Birkbeck College, University of London, Malet Street, London WC1E 7HX

18 suppressors of *alcR*125 have been isolated by T. J. Roberts (unpublished results). We have examined them to see whether they constitute a different group of suppressors from those previously isolated. Suppressors were characterised for their growth rate, cryo sensitivity, antibiotic sensitivity, morphology and suppression of *alX*4, *sB*43, *alcR*125 and *niaD*501. They were crossed to existing suppressors *suaA*101, *suaC*109, *suaD*103 and *suaB*111 to see whether they mapped in these or new loci. Some were mapped to specific linkage groups by haploidisation.

Fifteen suppressors had phenotypic characteristics also typical of ribosomal mutants, *i.e.*, hygromycin sensitivity or cold sensitivity together with slow growth. Three suppressors were essentially wildtype for these characters. The suppressors can be tentatively assigned to the categories—ribosomal suppressors and tRNA suppressors respectively.

All the suppressors have been mapped to linkage groups III or VII. One, similar to *suaB*111, maps at this locus. One maps at *suaA*, eight are linked to *suaB* but suspected to be *suaA*, four are possibly *suaC*, two are unclassified and two map new loci on III. Seven ribosomal type suppressors are recessive and one tRNA type suppressor is semi-dominant.

On the basis of *in vivo* suppression assessments, the suppressors are different from one another and from previously isolated ones.

Although many of these *alcR*125 suppressors map at known loci, as a class they include novel types of suppressor.

RECOMBINATION DEFICIENT STRAINS OF *USTILAGO MAYDIS*

R. HOLLIDAY AND S. Y. TAYLOR

National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA

Two radiation-sensitive mutants of *Ustilago maydis*, *rec*-1 and *rec*-2, have been previously characterised. Each is defective in radiation-induced mitotic recombination in heteroallelic diploid strains, whilst retaining spontaneous recombination. Diploids have been constructed which are homozygous for both *rec*-1 and *rec*-2 and heteroallelic for three auxotrophic loci. Spontaneous recombination is abolished at two of the loci and greatly reduced at the third.

rec-1 has a pleiotropic phenotype with effects on meiosis, chromosome stability and mutation, as well as on mitotic recombination and repair (Holliday *et al.*, *Genet. Res.*, *27*, 413–453, 1976). It is known to lack a DNA-binding protein with properties very similar to the *recA* protein of *Escherichia coli* (Kmiec and Holloman, *Cell*, *29*, 367–374, 1982). The protein can catalyse the formation of heteroduplex DNA from double- and single-stranded homologous sequences. *rec*-2 prevents the completion of meiosis, but its biochemical defect is unknown. *rec*-1 and *rec*-2 are probably blocked in different repair pathways, since double mutant strains are much more radiation-sensitive than either single mutant. It is concluded that this double block also produces a rec^{-} phenotype.

COMPETITION DIALLEL ANALYSIS OF MALE COURTSHIP BEHAVIOUR IN *DROSOPHILA MELANOGASTER*

MIKE F. COLLINS AND JOHN K. HEWITT

Department of Psychology, University of Birmingham, PO Box 363, Birmingham B15 2TT

The courtship sequence of *Drosophila melanogaster* is amenable to a time-sampling technique which permits detailed biometrical genetic analyses of the individual components of the male courtship sequence. A 4×4 competition diallel analysis of male courtship was performed; this consisted of measuring the courtship of all sixteen combinations of male and female genotypes using this time-sampling technique. The fifteen degrees of freedom for these genotype combinations may be split into four components: variation between males (3 df), variation between females (3 df), one degree of freedom for comparing the courtship within inbred lines (the leading diagonal, LD) and the courtship between inbred lines (the off diagonal, OD) and eight degrees of freedom for residual variation (mainly the interaction of the male and female genotypes). The results indicate that all components of the courtship sequence except the copulation index show significant male and female effects. The mean square between males is larger than that between females, which indicates that the role of the male is more important in the control of the variation in the courtship sequence than that of the female. The residual variation, which reflects an interaction of male and female genotype, is significant for all courtship measures except that of licking and attempted copulation. The significant LD/OD comparison for the overall courtship index, wing vibration index and copulation index indicates that males and females of the same genotype perform more successful courtship than the different combinations of male and female genotypes. The implications of these results will be discussed.

METHODS FOR TEACHING CHROMOSOME BEHAVIOUR AND RECOMBINATION OF DNA

B. W. BAINBRIDGE AND S. ELLIOTT

Department of Microbiology and EAR Unit, Queen Elizabeth College, Campden Hill Road, London W8 7AH

Routine methods for teaching undergraduates about the intricacies of chromosome and DNA movement include diagrams in textbooks and drawings on the blackboard. These suffer from showing still shots of what is in reality a continuous process of movement. Films of mitosis and meiosis can compensate to some extent for this but they have the disadvantage that gene exchange cannot be shown. Models can be constructed either by the lecturer or student to illustrate various proceses.

Commercial kits are available but adequate systems can be developed using Plasticine, popper beads and small magnets. There can be little doubt that most students learn more efficiently when they construct their own models of, for example, the molecular mechanism of recombination between DNA molecules. However it is difficult to illustrate more complex recombination of a series of genes using these models.

We have recently been experimenting with techniques for producing animated video-film of chromosome behaviour. Early methods used 35 mm transparencies and a slide synchroniser but problems were encountered with precise registration of pictures so that movement was uneven. The most successful method has been to prepare diagrams on clear cellulose acetate sheets and then to video-record these in sequence onto a master tape. On a sophisticated editing machine, *e.g.*, at the London Audio-visual Centre, it is then possible to edit the sound track, chromosome movement and inserts of a variety of photographs and lettering.

An example of a video-range illustrating recombination of DNA in bacteriophage lambda will be shown. Copies of this tape will be made available and further tapes are planned in future.

OPTIMISING HUMAN CHROMOSOME SEPARATION BY FLOW SORTING

PETER HARRIS, E. BOYD AND M. A. FERGUSON-SMITH

Duncan Guthrie Institute of Medical Genetics, University of Glasgow

In recent years, fluorescence activated cell sorters (FACS), have been used to sort individual human chromosomes from which the DNA has been extracted and used for gene

mapping by Southern blotting, and for the construction of chromosome specific libraries. A limitation exists in the number of human chromosomes which can be sorted free from significant contamination of other chromosomes. We have investigated the use of chromosome variants and rearrangements in conjunction with a variety of fluorescent stains for optimising chromosome separation.

Ethidium bromide stains DNA uniformly, giving a flow karyotype of about 20 peaks in normal human cells. Other stains bind preferentially to certain DNA bases, notably, Hoechst 33258 to AT bases and chromomycin -A3 to GC bases. Human chromosomes contain different proportions of AT and GC bases, so flow karyotypes produced with these base specific stains show different profiles from those with ethidium bromide, allowing some similarly sized chromosomes to be sorted separately.

The occurrence of centric heteromorphisms in certain individuals can alter the DNA content of specific chromosomes sufficiently to allow them to be sorted free from other chromosomes. Deletions and duplications can also move chromosomes into more sortable positions in the flow karyotype, or move a chromosome peak away from the chromosome to be sorted. Translocations are especially valuable in sorting experiments as they can be used for regional mapping of genes or cloned DNA fragments.

GENE CONVERSION AS A FORCE THAT CAN CHANGE DNA BASE RATIOS (GC CONTENT) AND TOTAL AMOUNTS OF DNA

B. C. LAMB

Department of Pure and Applied Biology, Imperial College, London SW7 2BB

Polymorphisms and heterozygosity are very common in many organisms. Disparity in the direction of meiotic gene conversion at heterozygous sites has been shown to be common for different types of mutation (base-substitutions and frame-shifts; spontaneous and induced); disparity favouring conversion to mutant (*m*) is about as frequent as disparity favouring wild-type (+) (Lamb and Helmi, *Genet. Res.*, *39*, 199–217, 1982).

A consideration of molecular causes of disparity in conversion direction for base-substitutions shows that most mechanisms giving disparity for $+/m$ also give disparity of base pairs resulting from conversion. Some mechanisms could have systematic effects on base ratios, over many sites, increasing or decreasing the GC content. Base-substitution mispairs in hybrid DNA comprise: purine/pyrimidine mispairs such as AC and GT from transitions (*e.g.*, AT to GC); unalike purine/purine and pyrimidine/pyrimidine mispairs from transversions (*e.g.*, AG and CT from AT to CG), and alike purine/purine and pyrimidine/pyrimidine mispairs from transversions (*e.g.*, AA and TT from AT to TA). With heterozygous frame-shifts, from base deletions or additions, mechanisms causing disparity of conversion direction for the non-pairs in hybrid DNA could cause systematic increases or decreases in total DNA, with or without changes in GC content.

Selection could counteract some such changes, but others would be in non-coding DNA or produce various kinds of cryptic mutation (Lamb, *Mol. gen. Genet.*, *137*, 305–314, 1975) not readily susceptible to selection. The rates of change would be slow but could be regulated by the properties of DNA-correction enzymes which show DNA-level selection.

EFFECTS OF ASPIRIN ON MITOCHONDRIAL MUTAGENS AND OLIGOMYCIN IN *SACCHAROMYCES CEREVISIAE*

IAN J. BRUCE AND DAVID WILKIE

Department of Botany and Microbiology, University College London, London WC1E 6BT

The mitochondrial mutation *petite* is induced in *S. cerevisiae* by ethidium bromide (EtBr), 4-nitroquinoline-1-oxide (NQO) and Adriamycin (ADR). The presence of aspirin in concentrations ranging from 0·1 to 1·0 mg/ml reversed, in a dose-dependent manner, the mutagenicity of EtBr and ADR but not that of NQO. Differences with respect to DNA reactivity between NQO on the one hand and EtBr and ADR on the other may be relevant in this distinction. Oligomycin is not mutagenic in this system but selectively blocks mitochondrial oxidative phosphorylation by virtue of its reactivity with the ATPase complex. This prevents growth of cells in non-fermentable media but growth is restored if aspirin is present, again in concentrations of 0·1–1·0 mg/ml. At these concentrations, aspirin stimulated mitochondrial

respiration in yeast cells in an oxygen electrode system but inhibited oxygen uptake in concentrations in excess of 2 mg/ml. At concentrations greater than 2 mg/ml, aspirin also induced the *petite* mutation. The mechanism of action of aspirin on mitochondria is discussed in terms of the drug's membrane-reacting and acetylating capabilities.

EXPRESSION OF Ty::*lacZ* FUSIONS IN YEAST

B. A. BOWEN, M. F. TUITE, A. M. FULTON, S. M. KINGSMAN AND A. J. KINGSMAN

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU

A *lacZ* gene fragment has been fused to a set of randomly generated sites at the 5′ ends of two Ty elements. In those molecules conferring a *Lac*+ phenotype *in vivo*, the *lacZ* fragment was fused in frame with an initiation codon residing in the 5′ delta. When a fragment containing 240 bp of the upstream delta sequence was deleted, *lacZ* expression was abolished, suggesting that both transcription and translation signals are present in each Ty. The copy number of *Lac*+ plasmids in a/α diploids was approximately double that in haploids, but the specific activity of β-galactosidase was reduced by up to 50 per cent. One other Ty::*lacZ* fusion 3 kb downstream from the 5′ delta was also *Lac*+. The activity of β-galactosidase in a/α diploids containing this fusion on a high copy number vector was depressed 10-fold.

MUTAGENESIS TO 2,6-DIAMINOPURINE RESISTANCE IN FRIEND LEUKAEMIA CELLS

P. G. McKENNA AND P. WARD

School of Biological and Environmental Studies, The New University of Ulster, Coleraine, Northern Ireland BT52 1SA

Clone 707 of the Friend murine erythroleukaemia cell line has an abnormally high mutation rate to resistance to the adenine analogue, 2,6-diaminopurine (DAP). DAP resistant Friend Cell mutants have greatly reduced ability to incorporate ^{3}H-adenine in their DNA, indicating that they may be deficient in the enzyme adenine phosphoribosyl transferase (APRT). Mutagenesis was carried out using the physical mutagen ultra-violet irradiation (UV), and the chemical mutagen ethyl methane sulphonate (EMS) in order to ascertain if the frequency DAP-resistance could be further enhanced by mutagenic treatment. A dose range of 1·2–3·6 J/m^{2}UV was used, and a clear dose-response was observed for mutations to both DAP-resistance and 6-thioguanine (6-TG) resistance. With EMS, a dose range of 100–300 μg/ml was used and again a clear dose-response was found for both the DAP-resistance and 6-TG-resistance markers. This indicates that DAP-resistance conforms to classical genetic predictions in its response to mutagenic treatment. Further mutagenesis experiments were carried out on wild-type (clone 707) cells and two DAP-resistant clones with 6-TG resistance being used as the marker. Both UV- and EMS-treatment yielded a higher mutagenic response in the DAP-resistant clones than in wild-type cells. This may indicate that APRT plays a role in balancing nucleotide pools, with absence of the enzyme leading to increased production of mutations during DNA repair processes. This research was supported by the Ulster Cancer Foundation.

A SCREENING SYSTEM FOR THE DETECTION OF ENVIRONMENTAL MUTAGENS EMPLOYING THE FISSION YEAST, *SCHIZOSACCHAROMYCES POMBE*

B. PATEL, J. F. T. SPENCER AND P. McATHEY

Department of Food and Biological Sciences, Polytechnic of North London, London N7 8DB and Department of Biological Sciences, Thames Polytechnic, London SE18 6PF

In recent years a high degree of correlation between mutagenicity and carcinogenicity has been established and this relationship has been exploited in the development of several bacterial systems, notably the Ames test (McCann, *et al.*, *Proc. Natl. Acad. Sci. USA*, *72*, 5135, 1975), designed to detect mutagenic activity (and hence predict carcinogenic activity) in environmental chemicals. We have developed a screening test for environmental mutagens employing the

fission yeast, *Schizosaccharomyces pombe*. This system, which involves monitoring induced mutation frequencies in continuous cultures, has a number of advantages over the conventional bacterial tests. Using our system, we have observed both the established mutagen ethyl methanesulphonate and certain potential mutagens to induce mutation to resistance to a number of antibiotics at several times the respective spontaneous mutation rates. The implications of these findings will be discussed.

MOLECULAR CLONING OF THE *cdc* 25$^+$ GENE OF *SCHIZOSACCHAROMYCES POMBE*

PETER FANTES AND JOYCE SHANNAN

Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT

Temperature-sensitive mutations at the *cdc* 25 locus have the following properties, suggesting that the gene may have a central role in the nutritional control of mitosis. (i) Cells are somewhat elongated at 25°C, the permissive temperature. (ii) After shift to 35°C, the cells stop dividing and arrest with a single G2 nucleus. (iii) Characters (i) and (ii) are phenotypically suppressed in a *wee* 1 genetic background, while *cdc* 2*w* (*wee* 2) mutations allow partial suppression (Fantes, P. A., *Nature, 279,* 428, 1979). (iv) *cdc* 25 mutations may affect the translational machinery of the cell since they act as allo-suppressors (Nurse, P. *et al., Heredity (Abstr.), 42,* 282, 1979).

We have undertaken the isolation of the *cdc* 25 gene by molecular cloning. A *cdc* 25·22 *leu* 1·32 strain was transformed with a genomic *S. pombe* library in the shuttle vector pDB248 (Beach, D. and Nurse, P., *Nature, 290,* 140, 1981). Prototrophic transformants were tested for growth at 35°C, and one strain able to grow was examined in detail. The ability to grow at 35°C was lost during vegetative growth under nonselective conditions, and this loss was coincident with loss of the *leu*$^+$ phenotype, suggesting that both characters were plasmid-borne. A plasmid was rescued in *E. coli* which conferred both *leu*$^+$ and *cdc*$^+$ characters when reintroduced into the original *S. pombe* strain.

Analysis of the plasmid sequences to date indicate the presence of a 9 kb insert containing 3 *Hin*dIII, 1 *Pst*I and 1 *Bam*HI restriction sites. The insert has been partially subcloned, and we are currently integrating the sequences into the *S. pombe* chromosome, to test by meiotic analysis whether the site of insertion is close to the *cdc* 25 locus. Future investigations will include estimation of the mRNA levels in *wee* and wild-type strains to test the hypothesis that suppression of *cdc* 25 phenotype is due to hyperexpression of the gene in *wee* strains.

CLONING OF THE *wee* 1$^+$ GENE OF *SCHIZOSACCHAROMYCES POMBE*

JILL E. OGDEN AND PETER A. FANTES

Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT

wee 1 mutations of *S. pombe* have a defect in mitotic control resulting in a reduced cell size at division. The *wee* 1$^+$ gene possibly codes for an inhibitor of mitosis that is diluted during cell growth to a level that permits cell division (Pritchard, R. H. *et al., Symp. Soc. Gen. Microbiol., 19,* 263, 1969). Molecular cloning of the *wee* 1$^+$ gene is of interest to determine whether there is periodic synthesis of the *wee* 1$^+$ gene product through the cell cycle.

As there is no direct growth selection for *wee* 1$^+$ strains, we have used the suppression of *cdc* 25 phenotype in *wee* 1$^+$ cells as our method of screening for the gene (Fantes, P. A., *Nature, 279,* 428, 1979). A *wee* 1·50 *cdc* 25·22 *leu* 1·32 (phenotypically *cdc*$^+$) strain was transformed with a *S. pombe* gene library constructed in the vector pDB248 (Beach, D. and Nurse, P., *Nature, 290,* 140, 1981). Of 7000 *leu*$^+$ transformants screened at 35°C, one showed restoration of the *cdc*$^-$ phenotype suggesting that the insert in the plasmid provided normal *wee* 1$^+$ function. The *wee* 1$^+$ transformant was mitotically unstable; loss of *cdc*$^-$ phenotype was coincident with loss of the *leu*$^+$ vector marker.

Analysis of total DNA from the transformant by Southern blotting and hybridisation with vector showed that plasmid sequences were present in very high molecular weight form. Similar analysis of restriction digests of total DNA suggested that the high molecular weight form

was composed of tandemly repeated monomeric units each comprising the vector with a 1·2 kb insert. Monomeric plasmid from the yeast transformant was recovered in *E. coli*. Reintroduction of plasmid monomer into the original *S. pombe* strain did not restore *cdc* 25$^-$ phenotype. The reason for this is unknown, but inefficient expression of the gene in monomeric compared with multimeric form may be responsible.

QUENCH CORRECTION OF INCORPORATED ^{14}C IN *ASPERGILLUS NIDULANS* COUNTED ON FILTER DISCS: A CAUTIONARY NOTE

A. G. BROWNLEE AND H. N. ARST Jr.

Department of Genetics, University of Newcastle Upon Tyne, Newcastle Upon Tyne NE1 7RU

We have developed a convenient method for ^{14}C quench correction for *Aspergillus nidulans* counted in liquid scintillator using the filter disc technique. By comparing intact and solubilised samples labelled to constant specific activity, a quench correction factor (QCF) was obtained for a range of sample sizes. Large differences in quenching, including sample self-absorption were found amongst ungerminated conidiospores and mycelia of different ages and also amongst strains of different genotypes. The correlation between sample dry weight and sample channels ratio of standards, once established, can be adapted to form a convenient means of determining sample size, and hence specific activity, by counting alone.

creB$^-$ and *creC*$^-$ mutations of *A. nidulans* have a carbon catabolite derepressed phenotype (Hynes, M. J. and Kelly, J. M., *Mol. Gen. Genet., 150,* 193, 1977) and certain growth properties suggest that reduced sugar uptake might be responsible (see Arst, H. N. Jr., *Symp. Soc. Gen. Microbiol.*, 31, 131, 1981). Uncorrected data from a study of D-[^{14}C] glucose uptake by mycelia of mutant strains suggests that uptake is defective; however, no such deficiency is apparent after applying QCE Conidiospores of mutant strains, likewise, have normal glucose uptake.

The method described should be suitable for other fungi and for other low energy beta-emitting nuclides.

CAPSID PROTEINS IN VLPs CONTAINING THE L AND M SPECIES IN KILLER YEAST

M. EL-SHERBEINI, D. J. TIPPER, D. J. MITCHELL AND K. A. BOSTIAN

Queen Mary College, London University, University of Massachusetts Medical School, Worcester MA, USA and Brown University, Providence RI, USA

Several groups have recently shown that at least two L-dsRNA species may coexist in Type 1 "killer" yeast. Field, *et al.*, (*Cell, 31,* 193, 1982) have differentiated two L species, L1 and La, by terminal sequence analysis. El-Sherbeini, Bevan and Mitchell (*Cur. Gen., 7,* 63, 1983) have shown the existence of two electrophoretically separable L species, termed "L_1" and "L_2". Sommer and Wickner (*Cell, 31,* 422, 1982) have differentiated three unique species, LA, LB and LC by T1 fingerprint and hybridisation studies. The latter two studies correlated the ability of a strain to maintain M_1-dsRNA with the presence of LA/"L_2". It has previously been shown that L-dsRNA genomes serve the maintenance function of providing capsid protein for M_1 encapsidation (Bostian *et al., J. Bact., 143,* 463, 1980).

We have now correlated "L_1" with LB or LC, and "L_2" with LA. These species of L have now been redesignated L_{1A} (≡LA and L_2) and $L_{1B/C}$ (≡LB, LC and L_1), subscript 1 indicating Type 1 killer yeast and A; B/C indicating alternative forms. Isolation of VLPs and dsRNAs from strains carrying various L species and comparison of the VLP capsid proteins and the *in vitro* dsRNA translation products indicate that each L species encodes a different capsid component, previously identified as ScV-P1 (L_{1A}) or ScV-P2 ($L_{1B/C}$) which correlates with the *in vitro* translation products L-P1 (L_{1A}, and L-P2+L-P3 ($L_{1B/C}$). Killer strains K382-23A and K19 both contain L_{1A}, $L_{1B/C}$ and M_1. Analysis of their VLPs suggests that L_{1A} and $L_{1B/C}$ are separately encapsidated in their homologous capsid protein while M_1 is encapsidated only by ScV-P1, the product of L_{1A}, confirming at least one reason for dependence of M_1 on L_{1A}. Both the L-dsRNA (L_2) and capsid protein of Type 2 killer strain Y1. 10 differ from those listed for Type 1 killers.

COMPARATIVE STUDIES ON THE L dsRNA GENOMES OF *SACCHAROMYCES CEREVISIAE* AND *YARROWIA LIPOLYTICA*

R. J. JEWERS, M. EL-SHERBEINI, H. A. MOUNTAIN, K. A. BOSTIAN, E. A. BEVAN AND D. J. MITCHELL

Queen Mary College, London University and Brown University, Providence RI, USA

Strains of *Yarrowia lipolytica* have been shown to possess a species of L size dsRNA genome (R. J. Jewers, *et al.*, *Abst. XIth Int. Conf. Yeast Genet. Mol. Biol.*, Montpellier, 1982; D. P. Groves, *et al.*, *Curr. Genet.*, *7*, 185, 1983).

Molecular weights of the $L_{1B/C}$ and L_{1A} genomes of *Saccharomyces cerevisiae* and the L_y genome of *Y. lipolytica* have been determined by formamide gel analysis using *Escherichia coli* rRNA as standards. The calculated M.Wts are as follows, *viz.*,

$$L_{1B/C} \equiv 3{\cdot}23 \times 10^6; \quad L_{1A} \equiv 2{\cdot}99 \times 10^6 \quad \text{and} \quad L_y \equiv 3{\cdot}29 \times 10^6.$$

It has been suggested that *Y. lipolytica* encodes a killer toxin (Groves, *et al.*). We have performed exhaustive cross-reaction plate tests on strains of *Y. lipolytica* and *S. cerevisiae* both with and without dsRNA genomes over the *p*H range 3·0–7·4 and have been unable to corroborate this result. Furthermore *in vitro* translation of M dsRNA of *S. cerevisiae* has been shown previously to direct the synthesis of a 32 K pretoxin (Bostian, *et al.*, *Nuc. Acid Res.*, *11* (4), 1077, 1983) whereas in our *in vitro* translations (see below) of the L_y genomes, no major polypeptide product of this size order is detectable.

It has previously been shown by various groups that L-containing capsids from *S. cerevisiae* possess RNA polymerase activity resulting in *ss* and *ss*, full-length RNA products (see review by Mitchell and Bevan, in *Yeast Genetics* ed. J. F. T. Spencer, *et al.*, Springer, NY, 1983). We have undertaken similar *in vitro* transcription experiments but using separated L_{1A} and $L_{1B/C}$ containing capsids and also L_y containing capsids from *Y. lipolytica*. We have demonstrated capsid-associated RNA polymerase activity for all three types of particle with both *ss* and *ds*, full-length RNA products.

In vitro translation of the L_y genome, using the reticulocyte cell-free protein synthesis system, resulted in the appearance of one major species, designated L_yP1 which co-migrates with L-P2 (≡ *in vivo* major capsid *pp* of $L_{1B/C}$—see previous abstract). Several minor translation products were also seen and one of these has been designated L_y-P2. The only two *in vivo* products (Y1V-P1) and (Y1V-P2) co-migrate, the former with L_yP1 and the latter with L_yP2. Peptide maps of the two *in vivo* products are identical, not only to each other but also to L_y-P1 and L_y-P2. Therefore, Y1V-P1 and Y1V-P2 are capsid polypeptides but the relationship between them is as yet unclear.

MAPPING OF *PHR*1, A YEAST PHOTOREACTIVATION GENE, FOLLOWING ITS CLONING AND CHROMOSOMAL INTEGRATION

D. SCHILD, C. CHANG, R. MORTIMER AND J. JOHNSTON

Department of Biophysics and Medical Physics, University of California, Berkeley and Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow

Photoreactivation is the direct enzymatic repair of pyrimidine dimers induced by ultraviolet light, in the presence of low wavelength visible light. *S. cerevisiae phr*1 mutants (Resnick, *Photochem. Photobiol.*, *9*, 307, 1969) lack the ability to repair UV damage by photoreactivation. The *phr*1 mutation has hitherto not been mapped.

We have isolated a plasmid derivative of YEp13, containing a 6·4 kb insert, which restores photoreactivation in, *phr*1 mutants (Johnston *et al.*, *Heredity*, *49*, 135, 1982). The restriction map of the insert suggests that the photoreactivation gene is the same as that cloned by Yasui and Chevallier (*Curr. Genet.*, *7*, 191, 1983). By partial *Pvu*II restriction resulting in some cutting solely within the insert, we have integrated our plasmid into a yeast chromosome at a site homologous to the insert. Using the method of Falco and Botstein (*Genetics*, in press), two independent integrants showed that this site of homology and integration was on chromosome XV. We then determined that the integrated photoreactivation gene was located approximately 13 cM from the gene *prt*1. Independently of gene cloning, we have shown that the *phr*1 mutation maps at this same location on chromosome XV. It is thus apparent that our plasmid insert contains the gene *PHR*1 and not a gene suppressing *phr*1.

Spontaneous integrants were also obtained and the integrated insert of one of these was mapped at a location approximately 7 cM from *ade*3 on chromosome VII. Since YEp13 carries

part of a Ty1 sequence, it is expected to occasionally integrate at chromosomal sites of Ty1 sequences. We have shown that *phr*1 does not map at the above location and therefore, tentatively, it is a probable site of Ty1 sequence or a solo δ sequence. Experiments which may confirm this proposal are in progress.

PEDIGREE OF THE YEAST GENETICS STOCK CENTER "WILD-TYPE" STRAINS S288C AND X2180 OF *S. CEREVISIAE*

J. R. JOHNSTON AND R. K. MORTIMER

Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow and Department of Biophysics and Medical Physics, University of California, Berkeley

The rapid expansion of the molecular genetics of yeast leads us to suggest that greater attention is due to the interrelationships of the strains used in studies such as gene cloning and sequencing. Various heterogeneities in laboratory strains have recently been reported, including those pertaining to the distribution of restriction sites and the numbers and positions of transposable elements and repeated gene families.

To allow better comparison of these heterogeneities relative to the strains in which they have been observed, we have constructed the pedigree of one of the most common progenitor haploid strains, S288C and its diploid derivative, X2180. The former was used to isolate almost all of the mutants of the arginine, histidine, leucine, lysine, methionine, threonine, tryptophan and uracil biosynthetic pathways. Combinations of these mutants provide most of the original strains for the Yeast Genetics Stock Center which has been in operation since 1960. We have determined which strains were used by such early yeast biologists and geneticists as Lindegren; Reaume and Tatum; Pomper and Burkholder, Ephrussi; Roman, Douglas and Hawthorne; and Zirkle, Tobias and Mortimer.

The pedigree of S288C reveals six ancestral strains. These are *S. cerevisiae* 93 and *S. carlsbergensis* 126 (isolated by E. Mrak, University of California, Davis in 1938); baker's yeasts (*S. cerevisiae*) FLD, LK (Lindegren, *The Yeast Cell*, 1949) and Yeast Foam (*Ephrussi, et al., Ann. Inst. Pasteur, 76,* 419, 1949); and *S. microellipsoideus* NRRL-210. We have found that the contribution of the heterothallic diploid strain 93 is paramount and have estimated that 80–90 per cent of the genome of S288C derives from this ancestral strain *via* its segregants 93-1C and 93-3B.

Cultures of strains 93 and 126 are still available at the University of California, Davis (H. Phaff) and the former strain was recently recovered by us from lypholyzed cultures made in 1951 at the University of California, Berkeley (RKM). Tetrad analysis of the latter culture shows that, in addition to mating type, strain 93 segregates for *gal*2, a gene for copper resistance (probably *CUP*1) and a gene for flocculation which is currently under investigation.

It is our opinion that strain S288C and hence ancestral strain 93 is a major source of the gene pool of a majority of strains currently in use in yeast genetics and molecular biology.