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1. Reverse-phase high-performance liquid chromatography of the 60S subunit proteins of the fungus *Coprinus cinereus*: evidence for an altered 60S protein in a cycloheximide-resistant mutant

J. Sardharwalla* and J. North

*City of London Polytechnic, Calcutta House, Old Castle Street, London E1 7NT. *Department of Biochemistry, King's College London (KQC), Strand, London WC2R 2LS.*

In vitro protein synthesis studies of cycloheximide-resistant mutant of *Coprinus cinereus* have shown that resistance to the drug is associated with the 60S subunit of the 80S ribosomes. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) has been explored as an approach for the separation, analysis and comparison of the proteins of the 60S subunits from the cycloheximide-sensitive and -resistant strains CY8 and CY8.2 respectively. Using a μ-Bondapak C_{18} column and a trifluoroacetic acid/acetonitrile solvent system, the proteins from this subunit have been resolved into approximately 27 peaks. Comparison of the protein chromatograms of the two strains showed two major differences: peak *X*, seen in the resistant strain, is almost completely absent in the sensitive strain; whereas peak *Y* is reduced considerably in intensity in the resistant strain. These differences correspond to the pattern shown after One-Dimensional Urea-Polyacrylamide Gel Electrophoresis (ID-UREA/PAGE) of these proteins. However, only 11 protein bands were resolved by the latter technique.

These results provide further evidence that resistance to cycloheximide is a property of the 60S subunit involving a protein component. They also clearly establish RP-HPLC as a method of choice for the rapid analysis and comparison of eukaryotic ribosomal proteins, offering far better resolution and reproducibility than ID-UREA/PAGE.

2. An analysis of the cytoplasmic ribosomal proteins of cycloheximide resistant and sensitive monokaryotic strains and heterozygous dikaryons of *Coprinus cinereus* using 2-dimensional P.A.G.E.

J. D. Traynor and J. North

Department of Biological Sciences, City of London Polytechnic, Calcutta House, Old Castle Street, London E1 7NT.

2-Dimensional polyacrylamide gel electrophoresis (P.A.G.E.) was used to study the cytoplasmic ribosomal proteins of *Coprinus cinereus*. The 40S ribosomal subunit had a complement of 24–30 proteins which are identical in cycloheximide resistant strain CY8.2 and its corresponding wild-type CY8. In contrast, the proteins of the 60S ribosomal subunits of these strains showed a number of differences, but comparison of these with 60S proteins from CY9.23 and CY9, another pair of strains resistant and sensitive to cycloheximide showed that only one protein, Ln, was consistently associated with resistance.

Analysis of 60S proteins from dikaryons heterozygous for resistance failed to confirm the role of Ln in resistance, but did reveal the presence of 9 extra proteins which were probably derived from the second parents used to make the dikaryons, CY3 and CY13, which have a different genetic background from CY8 and CY9. In addition, there were 5 proteins identified in the monokaryons which were absent from the dikaryons in a pattern suggesting some type of regulation.

In all, 62 proteins were observed in the large subunit, the range associated with any one strain being 38–50.

3. Cloning of the *arg-12* gene and cross-pathway regulation of *arg-12* mRNA in *Neurospora crassa*

H. J. Flint*, J. Wilkening and I. B. Barthelmess****

**Department of Genetics, King's Buildings, University of Edinburgh, Edinburgh EH9 3JN; **Institut für Angewandte Genetik, Universität Hannover, Herrenhäusesstr. 2, 3 Hannover 21, West Germany.*

Ornithine carbamyl transferase (OCTase), which is encoded by the *arg-12* locus, is one of many amino acid synthetic enzymes whose activity is regulated through cross-pathway amino acid control in *Neurospora crassa.* This enzyme is encoded by the *arg-B* locus in *Aspergillus nidulans* (Berse *et al.*, *Gene* 25, 109). Probes derived from the *arg-B* gene have now been used to identify and clone a partially homologous fragment from *Neurospora* genomic DNA that carries the *arg-12* gene.

arg-12 DNA probes hybridise to a single poly A^+ transcript whose concentration was found to increase greatly *in vivo* under conditions of amino acid limitation that elicit cross-pathway derepression of OCTase activity. This response was abolished in *cpc-1* mutant strains that are impaired in cross-pathway regulation, showing that the product of the *cpc-1* locus is involved in controlling OCTase mRNA levels, probably via the rate of transcription. The level of NADP glutamate dehydrogenase mRNA (coded by the *am* gene—Kinnaird *et al.*, *Gene* 26, 253) was unaffected either by amino acid limitation or by the *cpc-1* mutation. We have also investigated the rapidity and kinetics of the response of OCTase mRNA levels to amino acid limitation in *cpc-1* strains, which have further implications for the regulatory mechanisms involved.

4. The analysis of sperm chromosomes in individuals exposed to genotoxic agents

P. T. Tomkins, C. V. Carroll, J. A. Houghton and M. Hurley*

*Microbiology Department, University College, Galway, Ireland. *Radiotherapy Department, Cork Regional Hospital, Cork, Ireland.*

Little is known about the congenital consequences of paternal exposure to mutagenic and clastogenic agents, despite the existence of two large amenable groups of individuals who have been so exposed:

(a) Men who have undergone chemotherapy and radiation treatment for cancer, and
(b) Atomic bomb survivors.

Studies of the latter groups have failed to demonstrate an increase in the frequency of birth defects or chromosome aberrations (Awa, In: *Radiation-induced chromosome damage in Man*, 1983, p. 433–453, publ. A.R. Liss). Evidence for the former group is far less copious.

We have applied a heterospecific *in vitro* fertilisation procedure, the sperm chromosome assay (SCA) (Tomkins *et al.*, *Heredity 50*: 211, 1983), to a number of men who are or were on individual or combined cytotoxic and radiation regimes. The majority of such individuals initially become azoospermic and those that recover spermatogenic function after treatment ceases, generally pass through an oligospermic phase. These samples behave poorly in the SCA and we have utilised specific sperm selection and capacitation techniques to combat this problem. Further, batch variation in human serum albumin and foetal calf serum used in the culture media also affect the efficiency and outcome of the SC. We have recently succeeded in conducting the entire assay in the absence of both of these components using fully defined media with a concomitant improvement in metaphase yield and quality. The advantage of this interventionist approach to the attainment and analysis of sperm chromosomes from genotoxic exposed oligospermic samples will be discussed.

5. Control of DNA synthesis in bacteriophage T1

C. L. A. Paiva, D. A. Ritchie and G. Hope*

*Department of Genetics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX. *Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR.*

The T1 genome encodes about 31 proteins, of which six are involved with DNA replication and recombination. Genes 1 and 2 are implicated in the initiation of T1 DNA synthesis; 2.5 in degradation of host DNA and shut off of host replication;

3.5 and 4 in the maintenance of viral DNA synthesis and genetic recombination; and *das* in the partial reversal of T1 "DNA arrest" phenotype caused by mutations in either gene 3.5 or 4.

Mutations in genes 3.5 and 4 produce a pleiotropic phenotype which includes: premature arrest of T1 DNA synthesis, failure to form concatemers, reduced recombination, reduced ATP independent 5′-exonuclease activity, production of shorter than unit length progeny DNA molecules.

We have identified a low molecular weight peptide (called X) which seems to be a strong candidate for the primary cause of the premature arrest of DNA synthesis observed for gene 3.5 and 4 mutations.

The possible roles of peptide X on the premature arrest of DNA synthesis and also its possible involvement with the control of replication in T1 will be discussed.

6. Molecular studies on the response of *Brassica campestris* to challenge by pathogenic and non-pathogenic mutants of *Xanthomonas campestris* pv. *campestris* and pv. *vitians*

D. B. Collinge, J. M. Dow, D. E. Milligan and M. J. Daniels

John Innes Institute, Colney Lane, Norwich NR4 7UH.

Xanthomonas campestris pv. *campestris* is the causative agent of black rot of Brassicas. Inoculation of mature leaves of *Brassica campestris* with *X.c.* pv. *vitians* (a pathogen of *Lactuca sativa*) causes hypersensitive reaction (HR) which is localised to the region of inoculation. Physiological and biochemical changes can be observed in inoculated areas which differ from those caused by virulent forms of *X.c.* pv. *campestris*. Various non-pathogenic mutants of pv. *campestris* have been identified which give responses which range from something similar to HR to no detectable response.

mRNA preparations have been prepared from *B. campestris* leaves inoculated with *X. campestris* pathovars and mutants, and *in vitro* translocations have been performed using these which indicate some differences in plant gene expression.

7. Biased inheritance of optional DNA insertions following mitochondrial genome recombination in *Coprinus cinereus*

A. Economou, M. E. Zolan, P. J. Pukkila and L. A. Casselton

School of Biological Sciences, Queen Mary College, Mile End Road, London E1 2EY and Department of Biology & Curriculum in Genetics, University of North Carolina, Chapel Hill, N.C. 27514, U.S.A.

Two variant mitochondrial DNAs in *Coprinus cinereus*, H and J, are distinguished by having alternative 1·23 Kb insertions at sites approximately 2 Kb apart in, or adjacent to, the *co-1* gene. Genetic recombination between J and H genomes nearly always results in generation of a genome that contains both these insertions. Recombination was detected using the mitochondrial gene mutations *acu-10*, which causes a cytochrome oxidase defect, and *cap-1* which confers resistance to chloramphenicol. In one series of crosses recombination gave rise to the wild type genotype *acu-10*$^+$ *cap-1*$^+$. Fourteen of 15 independently derived recombinants were found to have both DNA insertions. In a second series of crosses intragenic recombination within the *cap-1* gene was detected. *cap-1* is assumed to be the structural gene for the LrRNA and is some 6 Kb distant from the nearest insertion site. Recombination within this gene must therefore be independent of any event leading to recombination of the insertions. Eight independently derived *cap-1*$^+$ recombinants were examined and in all both DNA insertions were present. Hybridisation studies have shown that the insertions are non-homologous. The biased inheritance of these sequences suggests that they encode a function similar to that of the ω^+ intron in the mitochondrial DNA of *Saccaromyces cerevisiae* and that we are observing analogous conversion events.

8. Ammonium ion toxicity and ribosomal mutations in *Aspergillus nidulans*

J. Hudson, R. Bratt and S. Martinelli

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

Ammonium tartrate or chloride are the most frequently used nitrogen sources in our laboratory.

A chance observation that some suppressor-containing strains grew poorly on ammonium medium but almost normally on nitrate medium has led us to screen all translational mutants for ammonium sensitivity.

Strains with mutations in the putative tRNA genes, *suaB* and *suaD*, grew like wild-type on ammonium medium whereas those strains with ribosomal suppressors (*suaA* and *suaC*) behaved abnormally. Several *suaC* alleles have been examined, and one of these, *suaC109*, has been tested in several genetic backgrounds All these strains are inhibited by nitrogen source. Increasing the ammonium concentration gives a more pronounced effect. This is in contrast to normal growth on proline, sodium nitrate or glutamate. Any ammonium salt has the same effect. Further antisuppressor mutations which partially compensate for the hypersensitive reaction of *suaC109* strains to antibiotics and low temperature also correct to various degrees the ammonium sensitivity.

Since the antisuppressor genes and *suaC* are thought to code for ribosomal proteins, one possible explanation is that the ammonium ions interfere directly with ribosome function or structure. Although magnesium is the element which springs to mind in the context of ribosomes, monovalent ions also play a part in the association (or opposite) of ribosomal subunits. We are examining the effects of ammonium and other ions, *in vitro*, on ribosome dissociation.

We are also trying to exploit the toxic effect of ammonium ions to isolate new types of ribosomal mutant.

10. Genetic controls of genome instability in *Escherichia coli* K-12

A. P. Jessop and F. Rodger

Institute of Genetics, University of Glasgow, Glasgow G11 5JS.

Homologous recombination not only brings about recombination in genetic crosses, but also acts on intrachromosomal homologous sequences to generate chromosome rearrangements. We present data on a *recA*-dependent instability in the 77′ region of *E. coli* K-12, where a genetic component in one of our strains dramatically increases the frequency of occurrence of the instability.

11. Identification of a cDNA clone for the cyanogenic β-glucosidase gene from *Trifolium repens* L. (white clover)

A. L. Sharif, E. Oxtoby, M. A. Dunn, D. B. Collinge and M. A. Hugh

Department of Genetics, University of Newcastle upon Tyne, Ridley Buildings, Newcastle upon Tyne NE1 7RU.

Activity of the white clover cyanogenic β-glucosidase, linarmarase, is controlled by the *Li* locus. Affinity purified antibodies raised to a denatured form of linamarase will immunoprecipitate a 59×10^3 Mr *in vitro* translation product from mRNA extracted from developing leaves of *Li Li* and *Li li* plants. No such polypeptide is produced when mRNA from *li li* genotypes is used. The 59×10^3 Mr nascent polypeptide is processed to a 62×10^3 Mr protein (the subunit molecular weight of native, active linamarase) by unwashed dog pancreas microsomes in a wheat germ *in vitro* translation system. A cDNA library was made in pBR322 using poly A^{++} mRNA templates extracted from developing leaf tissue, genotype *Li Li*. Putative linamarase clones were selected by differential colony screening using ss cDNA probes synthesised using either *Li Li* or *li li* mRNA as a template. A linamarase clone was identified amongst the putative clones by the hybrid select translation of a 59×10^3 Mr polypeptide which is immuno-precipitated by affinity purified denatured—linamarase antibodies.

12. A cell-free system for examining ribosomal mutants in *Aspergillus nidulans*

A. Zamir and S. Martinelli

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

A cell-free system has been developed for synthesising polypeptides *in vitro*, using extracts of *A. nidulans*. Since it is an homologous system, it can be used for studying translation mutants. The system is a modified version of that used for *Podospora anserina* by M. Picard-Bennoun and colleagues. For this work we have to purify the elongation factors partially from the S100 extract. The factors are very labile and have to be used quickly.

Preliminary studies have been made on wild-type ribosomes and their susceptibility to ribosomal antibiotics which inhibit growth of *Aspergillus*. Cycloheximide, an inhibitor of the large sub-unit simply and dramatically inhibits polypeptide chain elongation. Several aminoglycoside antibiotics have a complex effect since they inhibit amino acid incorporation at low concentrations and stimulate it at higher concentrations. This effect is being studied in conjunction with an assay for misincorporation.

Several antibiotic resistant mutants have been examined, *in vitro*, without finding any with altered ribosomal resistance. However some ribosomal suppressor mutants have ribosomes with altered sensitivity to aminoglycoside antibiotics, both *in vitro* and *in vivo*.

13. Recombination of mitochondrial genomes in *Coprinus cinereus*: its physico-chemical characterisation

J. L. Baptista-Ferreira and R. Vidal Silva

Centro de Micologia da Universidade de Lisboa, Faculdade de Ciencias, 1294 Lisboa Codex, Portugal.

Dikaryon formation, the production of sexual mycelium from two compatible monokaryons in the basidiomycete *Coprinus cinereus*, is achieved by nuclear migration independent of cytoplasmic movement; as a result, the reciprocal dikaryons formed at the periphery of the opposed monokaryons only have the resident cytoplasm. However, it was shown (Baptista-Ferreira, Ph.D. Thesis, University of London, 1981) that cytoplasms mixed as a result of hyphal anastomoses and suggested that recombination, rather than heterozygosity, of cytoplasmic genes occurred in the zone of contact of mated monokaryons.

Using appropriate mitochondrial gene mutations carried in compatible monokaryons, it was shown, from evidence produced by somatic segregation analysis, that recombinant mitochondria arise in the area where mated monokaryons had anastomosed (Baptista-Ferreira, Economou and Casselton, *Current Genetics* 7, 407, 1983).

An investigation at the molecular level by physico-chemical analysis of the mitDNA is currently underway and the results are presented.

14. Electrophoresis of the ribosomal proteins of translation mutants of *Aspergillus nidulans*

R. Bratt and S. Martinelli

Department of Botany, Birkbeck College, University of London, Malet Street, London WC1 7HX.

Using the "4 Corner Method" of electrophoresis for proteins developed by Madjar *et al.* (*Anal. Biochem. 92*, 174, 1979) we are analysing the protein components of cytoplasmic ribosomes of the fungus *Aspergillus nidulans*. Using strain *biA1* (Glasgow) as wild type the 40S, 60S and 80S proteins have been electrophoretically separated. Most of the proteins can be assigned to a sub-unit but others are as yet uncertain.

Strains containing translational suppressor mutations are being compared with isogenic strains and results will be presented which show that they have altered electrophoretic migration patterns in a protein. Mutations at the *suaA* and *suaC* loci (Roberts *et al.*, *Molec. gen. Genet. 177*, 57, 1979) were used for this study.

15. Biochemical genetics of nitrate assimilation in yeast

C. P. Jones, J. L. Wray and J. R. Kinghorn

Department of Biochemistry and Microbiology, University of St. Andrews, Irvine Building, North Street, St. Andrews, Fife, Scotland KY16 9AL.

The nitrate-utilising haploid yeast, *Hansenula wingei* offers the rare opportunity to study the biochemical genetics of nitrate assimilation in yeasts. We isolated spontaneous nitrate assimilation mutants in strains (ATCC 28162 and SH-00016) of opposite mating type, at an overall-frequency of 20 per cent, by selecting for resistance to chlorate in the presence of glutamate as sole nitrogen source. Four classes of chlorate-resistant mutant were found, viz:

Class I: nitrate non-utilising, nitrite utilising;
Class II: both nitrate and nitrite non-utilising;
Class III: nitrate utilising, nitrite non-utilising;
Class IV: nitrate and nitrite-utilising.

Class III mutants were unstable and reverted to either Class II or Class IV phenotype. The Class III phenotype has not previously been described in any organism. Genetic analysis revealed a number of complementation groups in Class I. All representative alleles of each complementation group lacked NR activity. Attempts to determine

which might be molybdenum cofactor (Mo-co) mutants were frustrated by the inability to assay xanthine dehydrogenase by any of the six previously published procedures. Although all could grow on hypoxanthine as sole nitrogen source some were Mo-co defective since (a) NR activity and growth could be restored in some cases, in the presence of unphysiologically high levels of molybdate and (b) the Mo-co of other groups was unable to reconstitute NADPH-NR activity in the *Neurospora crassa nit-1* reconstitution assay. Class II contained three inter-allelic complementation groups which had low or zero NR and NiR activity as well as low levels of functional Mo-co. We suggest that these three groups may represent a complex locus involved in the regulation of NR and NiR and perhaps also the Mo-co.

16. Molecular analysis of the complex *arom* locus in *Aspergillus nidulans*

I. G. Charles and A. R. Hawkins*

*Department of Biochemistry, University of Leicester, Leicester LE1 7RH; *Department of Genetics, Ridley Building, University of Newcastle on Tyne, Newcastle on Tyne NE1 7RU.*

The *arom* locus of *A. nidulans* and other filamentous fungi encode a multi-functional polypeptide that catalyses five consecutive enzymatic steps leading to chorismic acid in the polyaromatic amino acid biosynthetic (*arom*) pathway.

Arom DNA sequence data will be presented and the evolutionary relationships between the sequence of the dehydroquinase activity and that of its isoenzyme, catabolic dehydroquinase (encoded by the *QutE* gene in the quinic acid utilisation gene cluster) discussed. Analysis of *arom* specific mRNA will be described.

17. Gene replacement/disruption in *Aspergillus* by cotransformation

T. Goosen, K. Wernars, L. M. J. Wennekes, C. A. M. J. J. van den Hondel* and H. W. J. van den Broek

*Department of Genetics, Agricultural University, Wageningen, The Netherlands. *Medical Biological Laboratory TNO, Rijswijk, The Netherlands.*

In our studies on the transformation of *Aspergillus nidulans* we observed that a very high proportion of the transformants (60–100 per cent) can incorporate other, non-selected plasmids which are present in the transforming DNA mixture. This high cotransformation frequency enabled the introduction into *A. nidulans* of cosmids, plasmids and linear DNA fragments carrying genes and sequences for which direct selection is impossible.

To illustrate that the cotransformation phenomenon can be applied to introduce *in vitro* modified genes at specific, pre-determined loci we used a model system based on the *A. nidulans trpC-E. coli lacZ* fusion gene (van Gorcom *et al.*, *Gene*, in press).

Experiments will be described which show that by cotransformation of *A. nidulans* the wild type *trpC* gene can be replaced by the *trpC-lacZ* hybrid gene, resulting in tryptophan auxotrophic colonies with a $lacZ^+$ phenotype which are easily recognised by their blue colour on X-gal containing medium. These *trpC* mutants can be retransformed to $TrpC^+$ using the wild type *trpC* gene.

A detailed analysis of this system will be presented.

18. Structure and regulation of the pyruvate kinase gene of *Aspergillus nidulans*. L. de Graaff

W. J. van den Broek and J. Visser

Department of Genetics, Agricultural University, Generaal Foulkesweg 53, 6703 BM Wageningen, The Netherlands.

The pyruvate kinase (*pki*) gene of *Aspergillus nidulans* was isolated from a genomic library of *A. nidulans* in bacteriophage λ by heterologous hybridisation with a 1·8 kb *Eco*RI fragment from the yeast gene (Kawasaki and Fraenkel, *Biochem. Bioph. Res. Comm. 108*, 1107, 1982). Analysis of the selected clones by Southern blotting revealed a 2·9 kb *Eco*RI/*Bam*HI fragment which exclusively hybridised with the yeast *pki* gene. This fragment containing the gene was subcloned, and sequence analysis data will be presented. With the 2·9 kb *Eco*RI/*Bam*HI fragment as a probe for the wild type gene, we were able to demonstrate that a *pki* mutant, which lacks the *pki* gene product, contains a 150–200 bp deletion. Pyruvate kinase activity is stimulated by growth on glycolytic substrates. The regulation of the gene expression is studied both at the mRNA and the gene product level.

19. Analysis of regulation signals in *Aspergillus*

R. F. M. van Corcom, P. Punt, W. E. Timberlake*, H. W. J. van den Broek, H. Pouwels and A. M. J. J. van den Hondel**

*Medical Biological Laboratory TNO, Rijswijk, The Netherlands; *Department of Plant Pathology, University of California, Davis, USA; **Department of Genetics, Agricultural University, Wageningen, The Netherlands.*

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

To test whether the *lacZ*-fusion system also can be used in *Aspergillus* we have constructed *in vitro* a fusion of the *E. coli lacZ* gene and the *trpC* gene of *A. nidulans*. The fused gene was cloned into a plasmid which contains an *A. nidulans* selection marker. Analysis of *A. nidulans* strains transformed with this plasmid shows that the *E. coli lacZ* gene is expressed as a functional protein.

Based on the *trpC-lacZ* fusion gene, a set of promoter-probe vectors was constructed to isolate and analyse transcription/regulation signals. These vectors contain a unique *Bam*HI site in three different reading frames in front of either the truncated *trpC-lazZ* fusion gene or the trancated *lacZ* gene.

Experiments are in progress to analyse the promoter region of glycolytic and galactose-induceable genes in *A. nidulans* and *Aspergillus niger*. The results of such will be presented at the meeting.

20. Expression of bovine chymosin by *Aspergillus nidulans*

D. Cullen, R. Berka, G. Grey, K. Hayenga, S. Norton, M. Rey and L. Wilson

Genencor Inc., South San Francisco, CA 94080, U.S.A.

We have constructed several vectors for expressing rennin in *Aspergillus nidulans*. The promoter, presumptive upstream activator sequence, and terminator of the *A. niger* glucamylase gene were functionally linked to the gene encoding prorennin, and inserted as a *Cla*I fragment into plasmid pDJB3 (Ballance and Turner, *Gene*, in press). Further modifications involved the addition of various secretion signals to the prorennin gene.

Transformants of strain G191 were selected by *pyr*4 complementation and found to secrete active rennin in submerged culture. The identity of rennin and prorennin was confirmed by Western blots of one and two dimensional acrylamide gels. The mechanism of processing may be autocatalytic or may involve endogenous proteases. Rennin was secreted by transformants grown on starch, xylose and glucose indicating that the *A. niger* glucoamylase promoter is not regulated in *A. nidulans*. Southern analyses of selected transformants show integration of the *Cla*I fragment, and, for some transformants, multiple copies were observed.

21. An *Aspergillus* DNA sequence giving a high frequency of unstable transformants

I. L. Johnstone and A. J. Clutterbuck

Department of Genetics, Glasgow University, Glasgow G11 5JS.

In an attempt to construct a vector which would replicate autonomously in *Aspergillus nidulans*, a gene bank in the pILJ16 *argB*$^+$ vector was screened for production of semistable transformants of an *argB2* recipient. One such transformant, on conidial subculture, gave rise to approximately equal numbers of ARG$^-$ and unstable ARG$^+$ colonies. DNA from this strain was able to transform *E. coli* at high frequency to ampicillin resistance, and was shown to include both integrated and free copies of a pILJ16-derived plasmid with a 6 kb insert. This plasmid transformed *Aspergillus argB2* strains at high frequency (3000–5000 transformants per μg DNA) to give further unstable ARG$^+$ colonies. Analysis of the plasmid showed it contains an *Aspergillus* DNA insert consisting of 3 kb inverted repeats flanking a 376 bp unique core. Using this plasmid as a probe in Southern blots, suggests that wild type *Aspergillus* probably contains one copy of the inverted repeat, plus a few copies of the single arms.

22. Genetic and molecular analysis of the quinic acid utilisation gene cluster in *Aspergillus*

A. J. Francisco Da Silva, C. F. Roberts and A. R. Hawkins*

*Department of Genetics, University of Leicester, Leicester LE1 7RH; *Department of Genetics, Ridley Buildings, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU.*

Analysis of non-inducible mutants combined in heterozygous diploid strains of *Aspergillus nidulans* has defined two genes, *qut*A and *qut*D, which regulate the expression of a closely linked cluster of three structural genes. The product of one regulatory gene, *qut*D, is an activator required for the expression of all three enzyme structural genes and that of the second gene, *qut*A, has the characteristics of a co-activator required for gene expression in the presence of quinic acid.

In order to test alternative molecular models of the regulatory mechanism, we have cloned the three enzyme structural genes and also *qut*D using probes from the homologous genes in *Neurospora crassa*. Features of the promoter and terminator sequences flanking the enzyme coding regions will be described with particular reference to the dehydroquinase gene (*qut*E) and in comparison with recent data from *Neurospora* (Dr N. H. Giles, personal communication).

23. Transcription and processing signals revealed in the nucleotide sequence of the phosphoglycerate kinase (PGK) gene in *Aspergillus*

J. M. Clements and C. F. Roberts

Department of Genetics, University of Leicester, Leicester LE1 7RH.

The 3-phosphoglycerate kinase (PGK) gene from *Aspergillus nidulans* has been isolated from a Lambda-phage genomic library using the equivalent yeast gene as a hybridisation probe. The PGK gene has been shown to be constitutively expressed at relatively high levels in comparison to other *Aspergillus* genes.

The nucleotide sequence of the entire gene and its 5′ and 3′ flanking regions, and the transcription start and polyadenylation site of the PGK mRNA have been determined. The gene codes for an open reading frame of 421 amino acids and is interrupted by two 57 bp introns both of which have similar putative splicing signals to those found in other filamentous ascomycetes. The PGK promoter has CAAT and TATA homologies which are generally found in more complex eukaryotes. Additionally pyrimidine rich regions of the promoter sequence share some similarities to genes of other filamentous fungi. PGK mRNA exhibits 3′ heterogeneity and the major polyadenylation site is positioned 16 bp beyond the eukaryotic consensus polyadenylation signal AAUAAA. Potential hairpin structures are also found in the 3′ non-translated region of PGK mRNA which may be important in transcription termination and polyadenylation.

24. The molecular analysis of morphogenesis in *Sordaria brevicollis*

D. J. Bond and S. J. Broxhome

Department of Genetics, University of Edinburgh, Edinburgh EH9 3TN.

The morphogenetic events which we have been analysing in the fungus *Sordaria brevicollis* are those associated with fruiting. Firstly, we have been concerned with understanding signals which initiate the morphogenetic pathway leading to protoperithecial production, and secondly we have been analysing the morphogenetic pathway itself. The ultimate objective is to understand how the developmental events leading to perithecial formation and ascospore production are synchronised and co-ordinated at the molecular level.

When growing on cornmeal agar colonies of *Sordaria* are barren, protoperithecia are produced only after growth is disturbed, usually following contact with the Petri dish wall. There is a similar edge effect when the fungus is grown on chemically defined Westergaard and Mitchell's medium. On Vogel's N medium, in contrast, a growing colony does produce protoperithecia. This morphogenetic difference is brought about by the different nitrogen content of the two media. The addition of ammonium ions to, or reducing the level of nitrate ions in, Westergaard and Mitchell's medium separately induces a Vogel's-like response with respect to protoperithecial production.

At the molecular level protoperithecial formation is characterised by low level production of several phase-specific proteins (Nasrallah and Srb, *P.N.A.S. 74*: 3831–4). Further development is triggered by fertilisation when some of the phase-specific problems are formed in increased quantities. The molecular events underlying the mor-

phogenesis have been analysed by 2-D gel electrophoresis and *in vivo* and *in vitro* labelling. Thirty-one proteins have been identified which are present at some stage during differentiation but are absent from the vegetative mycelium.

25. Dominance and epistasy in the context of translation mutants of *Aspergillus nidulans*

S. D. Martinelli

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

Geneticists have used dominance tests as evidence when deciding whether mutations are in structural or regulatory genes and whether regulatory proteins are positively or negatively acting entities. In the context of translation, dominance tests have been used to assign mutations to tRNA genes (if dominant) and ribosomal genes (if recessive). The arguments employed to assign mutants in this way were based on assumptions of the molecular behaviour of the translation apparatus as well as the frequency of mutations at the locus in question and number of mutational sites. There are now an overwhelming number of contradictions with recessive tRNA mutations and dominant ribosomal mutations.

It follows that, my colleagues and I have also performed this ritual, with suppressor and antisuppressor mutations and have looked at all the aspects of their phenotypes, not only suppression. These results will be presented and the question posed "Are dominance tests worthwhile"?

By synthesising double mutants and then examining the epistatic relationships it is often possible to work out the respective positions of the two relevant genes in a metabolic pathway. I have made strains doubly mutant for a range of translational mutations and examined them in the hope that either predictions could be made about order of use during translation or ribosome synthesis, or about the nature of the suppressors concerned. The results are both interesting in showing up mutual antagonism and disappointing in that the character of the double mutant at low temperatures is usually similar to the weakest of the two partners, an effect that I also found with some developmental mutants years ago. Even so, I hope to be able to show at the meeting that the synthesis of double mutants is potentially more rewarding than that of dominance tests.

26. The repair of alkylation damage in *Aspergillus nidulans*

R. Swirski, B. M. Faulkner and P. Strike

Department of Genetics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Adaptation to the killing and mutagenic effects of simple alkylating agents in *Escherichia coli* has been shown to be due to the induction, respectively, of a DNA glycosylase (the *alkA* gene product), and an O^6-methyl guanine DNA methyltransferase (the *ada* gene product).

To determine whether such an inducible system existed in the lower eukaryote *Aspergillus nidulans*, conidia were pretreated with low concentrations of MMS and then challenged with a higher dose. The pre-treatment did not result in an enhanced ability to survive the challenge dose. However, comparisons of reversion frequency between adapted and non-adapted conidia revealed a three-fold reduction in the pre-treated cells following a high dose challenge.

Correlating these observations, and using suitable substrates and protein purification procedures, we have demonstrated that both a 3-methyl adenine glycosylase and an O^6-methyl guanine DNA methyl-transferase activity can be detected in such extracts. However, both of these activities appear to be constitutive, and are not affected by pre-treatment of mycelia with non-lethal levels of mutagen.

Several mutants displaying increased sensitivity to MNNG have been isolated, and mapped on the *A. nidulans* chromosomes. The most sensitive of these, SA1, is many more times more sensitive than wild type to NTG, but is not affected by UV light. A less sensitive mutant, SA3, is similarly unaffected by UV. These mutants, which map to chromosome VIII and I respectively, therefore appear to be defective specifically in steps concerned with the repair of alkylation damage.

27. Molecular mechanisms of heavy metal tolerance in *Aspergillus nidulans*

R. N. Cooley, D. A. Thurman* and A. B. Tomsett

*Department of Genetics and *Department of Botany, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.*

The response of the ascomycete fungus, *Aspergillus nidulans*, to a variety of metals has been tested.

Significant variation occurs between wild-type strains in the extent to which their hyphal growth is inhibited by metal ions. Less variation is seen in their inhibition of germination. Mutants have been isolated which are resistant to the presence of metal ions as shown by their increased ability for hyphal growth. The evidence suggests that the resistance of many of these mutants is not due to reduced rate of cellular uptake, either by extracellular chelation or by alteration in the rate of metal transport. Instead, the resistance may result from the induction of an intracellular mechanism, since a growth phase seems necessary for the resistance to become apparent.

Cadmium-resistant mutants have been mapped and found to be located at two loci. One locus is on chromosome VI between the *nicC* and *lacA* loci; the other is on chromosome IV between the *hisA* and *inoB* genes. The proximity of the chromosome VI mutants to the *methB* locus is of particular interest due to the relationship of sulphur-containing compounds and metal resistance.

Certain strains isolated as resistant to cobalt ions produce small spherical cobalt precipitates in the medium. In addition, a Cu-binding protein of similar size to yeast and animal metallothioneins has been identified.

28. Towards a molecular approach for dissecting the complex mating-type factors of the basidiomycetous fungi

Y. Parag

Department of Genetics, The Hebrew University, Jerusalem, Israel 91904.

The mating type (MT) genes in fungi are master-switches of the developmental events leading to initiation and sequential steps of the sexual cycle. In filamentous Ascomycetes and yeasts there is one MT locus with two alternative states; in the latter, the frequent conversion of one state to the other is well understood in molecular terms. In the Basidiomycetes the genetic control is much more complex. In *Schizophyllum commune* two mating-type (MT) factors, A and B, each comprises two discrete genes, α and β, each with a multiple allelic series. The combination of α and β alleles determines the mating specificity of each MT factor and a mating between homokaryons carrying compatible A and B MT factors lead to a normal dikaryotic mycelium on which fruiting bodies develop. The turning on/off of the master switches is executed through the exact recognition between specific combination of alleles. The B MT factors are grouped to phenotypic classes. Six mutations in one A factor and 27 mutations in eight B factors all resulted in the loss of mating specificity. Certain MT mutations or combinations of mutations lead to homokaryons mimicking dikaryons carrying fruiting bodies. Efforts to obtain mutations to new specificities using effective methods and mutagens have significantly failed in samples exceeding 10^9. The importance of a new level of analysis of MT factors by the use of gene cloning techniques is emphasised. The availability of (a) all the natural Bα and Bβ alleles, (b) almost all possible B factors, (c) large series of primary and secondary B mutations, (d) a deletion of the whole B region—all this accumulated knowledge renders the B factor not only an important target for the gene-cloning, but an effective candidate for such a study. Parameters—genetical, cytological and chemical concerning the genome of *Schizophyllum*, relevant to planning a strategy for cloning: 11 chromosomes including 7 mapped linkage groups with estimated genome size of 2000 CO units; some short distances involving MT genes: Bα-Bβ, class II = 2·5 kb, class III = 4·5 kb; Aα-pab = 8 kb. Methods for the use of the MT genes as selective markers and the phenotypes expected from this gene cloning are discussed.

29. An assessment of double-strand gap repair as a cause of gene conversion in fungi

B. C. Lamb

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

Many models have been proposed to explain gene conversion in fungi. "Gene conversion" is used here to include production of 5:3 and 3:5 ratios (+:*m*), as well as 6:2 and 2:6 s. In addition to hybrid-DNA (hDNA) theories, there is now the double-strand gap repair model (Szostak *et al.*, *Cell 33*, 25, 1983; Orr-Weaver and Szostak, *Microbiol. Rev. 49*, 33, 1985).

The proposed repair of double-strand gaps does not produce 5:3 s, 3:5 s aberrant 4:4 s or correction 4:4 s, but just gives 6:2 s and 2:6 s, probably in equal proportions. Within a length of gap repair, there should normally be no effect of

mutational type on conversion properties and one heterozygous mutation should not influence the conversion properties of nearby sites. One can therefore make detailed predictions about properties of gene conversion for situations with conversion: (a) almost exclusively from double-strand gap repair; (b) mainly from gap repair, but sometimes from hDNA; (c) sometimes from gap repair but mainly from hDNA; (d) almost exclusively from hDNA. Data from fungi can then be compared with the predictions to assess the importance of double-strand gap repair as a cause of gene conversion. In all cases, even (d), double-strand gaps could *initiate* gene conversion processes; what is being tested is whether actual conversions largely arise from gap repair or from hDNA.

Gene conversion data from *Ascobolus immersus*, *Sordaria fimicola* and *Sordaria brevicollis* generally fit predictions from situation (d) much better than those from situation (a), although situation (c) is not ruled out. Even data from yeast, which were originally quoted as supporting the double-strand gap model, fit predictions from (c) and (d) more often than they fit those from (a) or (b).

30. Polarity in *in vitro* recombination catalysed by cell extracts

P. Unrau, J. D. Childs, R. Aubin and M. Weinfeld

A.E.C.L. Radiation Biology Branch, Chalk River, Ontario, Canada K0J 1J0.

Plasmids used for *in vitro* recombination studies (Symington *et al.*, *Cell 35*, 805, 1983) were modified to permit the detection of polarity in recombination. Reciprocal crosses were made between plasmids with the mutations *tet*10 and *tet*14, located at 23 and 1246 bp respectively in the *tet* gene. Polarised recombination was shown by the preferential conversion of the *tet*10 mutation to wild type. Mixed plasmid clones arising from recombination were frequently observed and these also showed the preferential conversion of the *tet*10 allele. A computer search of the pBR322 genome located a 13 base sequence identical to one arm of the site—specific recombination sequence loxP of P1 phage (Hoess *et al.*, *P.N.A.S. 79*, 3398, 1982). Within this sequence a 15 base palindrome containing the TATAA region of the B-lactamase gene was identified. A deletion of this palindrome has been made to test the hypothesis that this region serves as a preferred site for initiation of recombination.

31. The analysis of the chromosomes of bovine sperm

P. Creighton and J. A. Houghton

Department of Microbiology, University College, Galway, Ireland.

A method developed in this laboratory for the analysis of human sperm chromosomes (Tomkins, Carroll and Houghton, *Heredity 50*, 211, 1983) based on the technique of *in vitro* fertilisation of zona-free hamster eggs described by Rudak *et al.* (*Nature 274*, 911, 1978) has now been extended to the visualisation of the chromosomes of bovine sperm. This communication describes recent improvements in this technique involving the use of follicular fluids and percoll gradients for the capacitation of the sperm. This method yields consistently analysable sperm metaphases with both fresh and frozen semen. Data from the study of these chromosomes will be described and the implications for cattle breeding and the A.I. industry discussed.

32. Cone pigment polymorphism and colour vision in the primates

H. Kalmus

Galton Laboratory, University College, London.

A recently described photopigment polymorphism in the New World Squirrel Monkey, *Saimiri sciureus* (Mollon *et al.*, *Proc. Roy. Soc.* B *222*, 373, 1984) differs radically from the genetical organisation of trichromatic colour vision in man and the Old World primates.

In the latter, alleles at two closely linked loci on the long arm of the X-chromosome separately control the photopigments of the red and green sensitive cones of the retina. In *Saimiri* three kinds of males and six kinds of females occur, of which only three—presumably heterozygotes—have trichromatic colour vision, all the others being dichromates. Mollon *et al.* explain that this represents a stable equilibrium, in which three alleles are maintained by female heterozygous advantage. In the retinae of these females, mosaics of two kinds of cones ("red" and "green" cones) occur owing presumably to lyonisation.

A derivation from some ancestral form of the human and *Saimiri* situations might be as follows: at some stage two alleles only existed at one locus; in the ancestors of *Saimiri* mutation then added a third, while in the ancestry of man gene duplication made subsequent differentiation possible. Such a scheme may shed a new light on the human colour vision deficiencies.

33. Incompatibility-induced cell death in *Phanerochaete velutina*

N. K. Todd, R. C. Aylmore, A. M. Ainsworth* and A. D. M. Rayner*

*Department of Biological Sciences, University of Exeter, Exeter EX4 4QG; *School of Biological Sciences, University of Bath, Bath BA2 7AY.*

Phanerochaete velutina possesses multinucleate hyphal compartments and whorled clamp-connexions. We have shown that it has a unifactorial (bipolar) mating system, anastomoses abundantly and exhibits a very rapid somatic incompatibility reaction at sites of hyphal fusion between genetically different secondary mycelia. In this paper, we present the cytological details of the somatic incompatibility reaction as seen using light and electron microscopy. Our observations prompt the speculation that incompatibility-induced cell death in this fungus occurs in a programmed manner.

34. The nucleotide sequence of the *Aspergillus nidulans gdh*A gene

S. J. Gurr and J. R. Kinghorn

Department of Biochemistry and Microbiology, University of St. Andrews, North Street, Irvine Building, St. Andrews, Fife KY16 9AL, Scotland.

The entire coding sequence of the *A. nidulans gdh*A gene for NADP-linked glutamate dehydrogenase (GDH) has been shown to reside within a 1·8 Kb HindIII fragment of DNA. From the nucleotide data, the deduced amino acid sequence was found to be highly homologous with other microbial GDH's particularly nearer the amino terminus and which is generally thought to be involved in catalytic activity and allosteric interactions. For instance, in the region amino acids 43 to 161 (*A. nidulans*) there is amino acid identity between *A. nidulans* and *N. crassa* (McPherson, M. J. and Woottom, J. C. *NAR 11*, 5257, 1983), *S. cerevisiae* (Nagasu, T. and Hall, B. D. *Gene* in press) and *E. coli* (Kinnaird, J. H. and Fincham, J. R. S. *Gene 26*, 253, 1983) of 88 per cent, 78 per cent and 79 per cent, respectively. Two short introns (55 and 56 bp respectively) interrupt these highly conserved *A. nidulans* sequences.

The *Aspergillus* introns are found at the precise positions as the two introns (66 and 61 bp) of the *N. crassa* glutamate dehydrogenase (*am*) gene. (The *S. cerevisiae gdh*A gene is not interrupted by intervening sequences). The internal sequences are not significantly conserved between *am* and *gdh*A introns. The two *gdh*A introns share an internal consensus, ACCGTTAA, not seen in other fungal or yeast introns, as well as an intact TACTAAC box in intron 2. Heterologous expression of *gdh*A has been demonstrated by transformation experiments using the *N. crassa* deletion mutant, *am*132. Information on the transcription initiation and polyadenylation sites, codon usage, and a comparison of *A. nidulans* GDH sequences with other available GDH's will be presented.

35. Transcription of the *Aspergillus nidulans* mitochondrial genome

N. J. Dyson, T. A. Brown, R. B. Waring and R. W. Davies

Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester M60 1QD.

Northern hybridisation, S1 mapping and *in vitro* capping experiments indicate that the *A. nidulans* mitochondrial genome is transcribed into RNA precursors that are rapidly processed into mature mRNAs.

We have mapped the 5'-termini of the 16S and 23S rRNA genes and located these in conserved nucleotide sequences that are similar to possible transcription initiation sequences in yeast. Loosely conserved sequences occur elsewhere in the *A. nidulans* genome, including at other possible transcription start points.

One major surprise is the presence of at least three mature multi-gene transcripts. These are relatively uncommon in the mitochondria of other lower eukaryotes. Evidence will also be presented for a possible role for tRNA genes in transcript processing, and for the involvement of RNA priming in genome replication.

36. tRNA genes: a transformation tool and a question about conversion

Y. Brygoo and R. Debuchy

Laboratoire de Génétique des Ribosomes, Bat. 400, U.P.S., 91405 Orsay Cedex, France.

Many suppressors restoring prototrophy were obtained from a strain *leu1-1* of the fungus *Podospora anserina*. Some, such as *su4-1* and *su8-1* were cloned in *Saccharomyces pombe* on the basis of the suppression of opal nonsense mutations.

Sequence analysis of the suppressor genes confirmed that they encode tRNAs reading UGA (opal), codon (Debuchy, R. and Brygoo, Y., in press). The sequence of the wild-type alleles revealed that both encode $tRNA^{Ser}_{UGA}$ with a low degree of homology. Furthermore, *su8* contains an intron, whereas *su4* does not.

As $Su4^+$ and $Su8^+$ encode isoacceptor tRNAs having the same anticodon their sequence differences are somewhat unusual. Intergenic conversion has been postulated as a mechanism for maintaining homology amongst members of a multigenic family, and, indeed interchromosomal gene conversion of $tRNA^{Ser}$ genes has been demonstrated in *S. pombe*. Thus, it is possible that the acquisition of an intron by one member of the ancestral family of $tRNA^{Ser}$ genes would have released this gene from the correction mechanism of gene conversion and allows a high degree of heterology to be maintained in *Podospora anserina*; the coding sequence differences observed between *su4* and *su8* may be correlated with the presence of an intervening sequence within *su8*.

The cloned tRNA suppressors were used in DNA transfection experiments with a strain *leu1-1* of *P. anserina* (Brygoo, Y. and Debuchy, R. *Mol. Gen. Genet. 200*, 128, 1985). Transformation occurs uniquely by integration. Genetic analysis showed that integration of the transforming molecule at the homologous site is not the rule: in most cases the integration point is located elsewhere. Some random integrations occur close to strategic loci as centromeres, which we hope to clone for constructing chromosome-like vectors.

37. At least seven ribosomal proteins are involved in the control of translational accuracy in the fungus *Podospora anserina*

M. Dequard-Chablat, E. Coppin-Raynal and M. Picard-Bennoun

Laboratoire de Génétique des Ribosomes, Unité Associée au CNRS No. 86, Bät 400, Université Paris-Sud, F.91405 Orsay Cedex, France.

In the fungus *Podospora anserina*, we have developed a genetic system to study the ribosomal control of translational accuracy (Picard-Bennoun *et al.* In: *Protein synthesis* (Abraham, A. K., Eikham, T. S., Pryme, I. F. eds), 1983). Ribosomal suppressors (*su*), analogous to "*ram*" mutations in *E. coli*, and antisuppressors (AS), analogous to bacterial "restrictive" mutations, have been characterised both genetically and biochemically. In *E. coli*, mutations which modify the error rate in translation alter the structural genes for 5 ribosomal proteins. So far 15 genes have been identified in *P. anserina*: 7 *su* genes and 8 *AS* genes. We have recently demonstrated that, at least, five of these genes are in fact structural genes for ribosomal proteins of the small subunit: S1 (*su*3), S7 (*su*12), S9 (*AS*9), S12 (*AS*1) and S19 (*AS*6) (Dequard-Chablat and Coppin-Raynal, *Molec. Gen. Genet. 195*, 294, 1984; Dequard-Chablat *et al.*, submitted for publication; Dequard-Chablat, submitted for publication; Dequard-Chablat, *Molec. Gen. Genet. 200*, 343, 1985). Two other ribosomal proteins are involved in translational accuracy: S29 which is lacking in all *AS*3 mutants and *S*8 which is altered in the single *su11* mutant characterised till now. One protein, namely *S7*, seems to play a key role in the ribosomal control of translational accuracy. Indeed, different alterations of this protein correspond to opposite effects. Forms more acidic than the wild-type lead to an increased accuracy, while more basic forms lead to decreased accuracy (Dequard-Chablat, submitted for publication).

38. J. R. S. Fincham

Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH.

39. Incompatibility genes regulating genes for fruit-body formation in *Schizophyllum commune*

J. G. H. Wessels

Department of Plant Physiology, Biological Centre, University of Groningen, The Netherlands.

Mating of two monokaryons of *Schizophyllum commune* carrying different alleles at both the *A* and

B incompatibility genes results in the formation of dikaryon with a typical mycelial morphology and the inherent capacity to form fruit-bodies under proper environmental conditions. Genetic analyses indicate that the incompatibility genes function as controlling genetic elements governing the expression of genes directly involved in morphogenesis. Till now we have not been able to detect mRNAs from genes involved in the monokaryon–dikaryon transition, possibly because their concentration is too low. However, during the formation of fruit-bodies by the dikaryon, a number of genes are uniquely expressed by producing abundant mRNAs and proteins. Probing the expression of these genes with cloned sequences revealed a low expression of these genes in the vegetative dikaryon but not in the monokaryons. It can thus be surmised that the regulatory role of the incompatibility genes in fruiting is the conditioning of fruiting genes in the chromatin in preparation for transcription, while full transcription of these genes is switched on by environmental signals conducive to fruiting.

40. Molecular genetics of pyrimidine metabolism in *Neurospora*

A. Radford, J. A. Glazebrook and S. F. Newbury

Department of Genetics, The University of Leeds, Leeds LS2 9JT.

We review the biosynthesis, uptake and salvage pathways of pyrimidines in *Neurospora*, and the loci involved. Regulation of the pathways by both nitrogen metabolite repression and pyrimidine-specific regulatory mechanisms will be discussed. The *pyr-4* gene, encoding orotidine 5′-P decarboxylase, has been cloned and sequenced. The results of sequence comparisons with the equivalent genes of *Saccharomyces cerevisiae* (*ura3*) and *Escherichia coli* (*pyrF*) will be presented and homologies discussed. The *pyr-4* clone has been inactivated by Tn1000 insertions, and also by removal of the distal end of the coding region and its replacement in a translational fusion by *lacZ*. Transformation of *Neurospora* with these defective clones, resulting in complementation to pyrimidine-independence has been achieved at low frequency. These results will be discussed in the context of transformation mechanisms in *Neurospora*.

41. Vectors for isolation of genes by transformation of *Aspergillus*

G. Turner

Department of Microbiology, University of Bristol, The Medical School, University Walk, Bristol BS8 1TD.

Genes can be isolated in *Aspergillus* by complementation of mutations using gene banks constructed in plasmid or cosmid vectors. The transformation frequency is generally low, but can be enhanced by inclusion of a chromosomal repetitive sequence *ans-1* into vectors. The nature and behaviour of this sequence in different vectors will be discussed, and its use in gene isolation.

42. Control of gene expression in *Aspergillus nidulans*

C. Scazzocchio

Institut de Microbiologie, Bat. 409, Université Paris-Sud Orsay Cedex.

Through the years the regulation of a number of dispensable metabolic pathways of *A. nidulans* has been studied thoroughly (reviewed in Arst, H. N. and Scazzocchio, C. in Bennet, J. W. and Lasure, L. L.; Gene Manipulations in Fungi, Academic Press, 1985, in press). A number of systems have been studied at the physiological level, structural and regulatory genes have been identified and in some cases very detailed fine structure maps are available.

We have cloned two complete systems, the ethanol utilisation regulon (Lockington *et al.*, *Gene 33*, 137, 1985) and a cluster comprising the three structural genes and the positive-acting regulatory gene involved in proline utilisation (Green, P., Scazzocchio, C., Arst, H. N. and Davies, R. W., unpublished). Northern blots confirm the supposition that the structural genes are regulated at the level of transcription and show that the cognate regulatory genes, *alc*R and *prn*A, are autoregulated. Interestingly *alc*R is directly regulated by carbon catabolite repression which seems to work by shutting off the synthesis of this positive-acting gene product rather than by repressing the expression of the structural genes themselves.

DNA sequencing of *alc*A and *ald*A, which are the genes coding for alcohol and aldehyde dehydrogenase respectively, has revealed highly conserved sequences 5′ to their coding regions, which might be involved in the regulatory process.

(Gween, D. I., Piggot, M. P., Davies, R. W., Lockington, R. A., Sealy-Lewis, M. H. and Scazzocchio, C., unpublished data).

43. The tubulin gene family of *Aspergillus nidulans*

N. R. Morris, G. May, J. Weatherbee, M. Tsang and P. Doshi

Department of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854, U.S.A.

All organisms that have been studied have multiple species of tubulin and multiple tubulin genes, but very little is known about the biological implications of this multiplicity of genes and proteins. *Aspergillus nidulans* provides an ideal experimental system for studying this problem since it has relatively few tubulins and tubulin genes; there are well characterised mutations in its tubulin genes; it has several differentiated cell types; and it is amenable to DNA mediated transformation.

A. nidulans has two genes for α-tubulin and two genes for β-tubulin, which together code for a total of six polypeptides. The *tubA* gene codes for two polypeptides, α1- and α3-tubulin. *Tub*B, codes for α2-tubulin. *Ben*A, codes for β1- and β2-tubulin, and *tub*C codes for β3-tubulin. The question is where and how do these genes and gene products function? *Ben*A and *tub*A are known to function in spindle and cytoplasmic microtubules of the vegetative mycelium. The following evidence indicates that *ben*A and *tub*C gene products are important for the development of asexual spores.

The two β-tubulin genes and one of the α-tubulin genes have been cloned and sequenced. The β-tubulin clones, B5 and B14, differ substantially with respect to nucleotide sequence and are only 80 per cent homologous with respect to protein sequence. Each integrates specifically in transformation experiments. B5 integrates at the *ben*A locus. Transformation with an internal fragment of B14 disrupts the *tub*C gene and causes the disappearance of β3-tubulin from 2D gels, indicating that β3-tubulin is the product of the *tub*C gene.

Asexual sporulation is normally sensitive to inhibition by benomyl in *ben*A22 (benomyl resistant) mutants. Disruption of β3-tubulin in a *ben*A22 strain by conidiation resistant mutations or by integration of a B14 internal fragment causes sporulation to become resistant to benomyl. This demonstrates that β3-tubulin functions during conidiation but is not required for conidiation. Since the ts *ben*A33 mutation blocks mitosis in conidiospores, β1- and/or β2-tubulin also must function during sporulation. Thus benomyl sensitive β3-tubulin confers benomyl sensitivity on conidiophores containing resistant β1- and/or β2-tubulin; but, when β3-tubulin is disrupted, the remaining resistant tubulins make sporulation resistant. These experiments demonstrate the power of the *Aspergillus* system for analysing cell specific functions of the tubulin gene family.

44. Position dependent and independent regulation of a gene from the SpoC1 gene cluster of *Aspergillus nidulans*

W. E. Timberlake, B. L. Miller, K. Y. Miller and K. Roberti

Department of Plant Pathology, University of California, Davis, California 95616, U.S.A.

We have previously described the structure and regulation expression of the SpoC1 gene cluster from *Aspergillus nidulans* (Gwynn, D. I., Miller, B. L., Miller, K. Y. and Timberlake, W. E., *J. Mol. Biol.*, *180*, 91, 1984). This 53 Kb segment of chromosomal DNA codes for at least 19 poly(A)$^+$ RNAs, some of which are transcribed from overlapping regions. The area of developmental regulation is approximately 37 Kb in length and is delimited by 1·1 Kb direct repeats. With one exception, RNAs transcribed from the central part of the cluster appear late during conidiophore development and accumulate specifically in spores. The exceptional transcript appears earlier during development and accumulates specifically in cells of the conidiophore. In contrast, RNAs encoded at the borders of the cluster occur in both somatic cells and spores. The results indicated that if a chromatin-level control mechanism operates to regulate expression of the SpoC1 gene cluster, as previously suggested by us (Timberlake, W. E. and Barnard, E. C., *Cell 26*, 29, 1981), additional levels of regulation must also exist.

We have now examined the effect of repositioning one SpoC1 gene, designated C1-C, in the genome. A recipient strain of *Aspergillus* was constructed containing a null allele of the C1-C gene. A plasmid containing a wild-type copy of the gene and its 5′ and 3′ flanking regions was then introduced by transformation. Several transformants were selected in which the plasmid had integrated at sites other than the SpoC1 region. Blot analysis

of RNA from these transformants indicated that the gene was expressed in somatic cells at a level much greater than when in the normal position. The repositioned genes nevertheless showed a considerable degree of developmental regulation. The results indicate that regulation of genes in the SpoC1 gene cluster involves both position dependent and position independent mechanisms.

45. H. N. Arst

Department of Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 0HS.

46. Lignin degradation and its control in *Phanerochaete chrysosporium*

P. Broda, O. Birch, A. Brown, R. Haylock, R. Liwicki, M. MacDonald, A. Paterson, U. Raeder, A. Schrank and P. Sims

Department of Biochemistry and Applied Biology, UMIST, Manchester M60 1QD.

In the white rot basidiomycete *Phanerochaete chrysosporium*, there is a switch determining whether or not lignin is degraded. Depletion of carbon or nitrogen sources results in increased adenyl cyclase activity and levels of cAMP, and the onset of ligninolytic activity. Of a series of phenol oxidase deficient mutants, some were unable to degrade lignin; of these some, but not others, had cAMP levels that remained low on C and N depletion. Others of these mutants degraded lignin more efficiently than the parent strain; these mutants had abnormally high cAMP levels on C and N depletion.

cDNA preparations were made from mRNA isolated from ligninolytic and non-ligninolytic cultures of the parent strain. These cDNAs were hybridised to genomic libraries of *P. chrysosporium* DNA, and a number of clones were isolated that carried sequences expressed only in the ligninolytic phase. These could be grouped into families by Southern blotting and by restriction site polymorphism segregation analysis (see Raeder and Broda, this meeting). Creation of a cDNA library allowed an assessment of the complexity of the mRNA population during lignin degradation.

Purification and partial sequencing of a ligninase protein allowed construction of oligonucleotides that were used to isolate from the gene library clones containing the corresponding gene. We seek to develop cloning systems allowing introduction of DNA into *P. chrysosporium*, and the progress towards this will be discussed. An objective is to obtain strains in which lignin degradation proceeds during normal growth.

This work is part of a programme supported jointly by British Petroleum's Venture Research Unit and the AFRC.

47. Studies on the structure and expression of cellulase genes from the filamentous fungus *Trichoderma reesei*

Jonathan Knowles, Päivi Lehtovaara, Tuula Teeri, Merja Pentillä and Irma Salovuori

Biotechnical Laboratory, VTT, SF-02150 Espoo, Finland.

Cellulases are a group of enzymes secreted by a number of different microorganisms all of which hydrolyse β-1,4 glycosidic bonds, though each has its own substrate specificity. We have cloned genes coding for 5 different cellulolytic enzymes and analysed 3 of these in detail. Full length cDNA clones have been isolated and characterised allowing the identification of at least two introns in each gene.

Comparison of the DNA and putative protein sequences of the genes coding for the cellobiohydrolases CBH I and II and endoglucanase EG I reveals a number of interesting features about the evolution of cellulases genes. It would seem that exon shuffling and both divergent and covergent evolution have played important roles in the evolution of these genes of this family.

48. D. A. Hopwood

John Innes Institute, Colney Lane, Norwich NR4 7UH.

49. B. A. Bridges

M.R.C. Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR.

50. Restriction site polymorphism segregation analysis (RSPSA) in *Phanerochaete chrysosporium*

U. Raeder and P. M. A. Broda

Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester M60 1QD.

We have developed a rapid method for preparing DNA from filamentous fungi and we have isolated

DNA from cultures derived from 50 independent basidiospores and the parent *P. chrysosporium* strain. Labelled DNAs from genomic clones (from a λ gene bank) were hybridised to Southern blots of *Sal*I restricted DNAs from the parent *P. chrysosporium* and the basidiospores. This showed that:
(a) about 80 per cent of the clones gave different hybridisation signals with parent DNA compared to basidiospore DNA, with 2 different types of patterns in the basidiospore DNAs (*i.e.*, there seemed to be segregation of "restriction site polymorphism");
(b) Therefore, the basidiospores seem to be haploid, whereas the parent *P. chrysosporium* strain contains two different homologous copies of most sequences and is probably dikaryotic;
(c) the RSP showed a 1:1 segregation pattern in the basidiospores as expected from meiosis.

RSPSA was used to investigate genetic linkage of genes specifically expressed during secondary metabolism of this fungus. This and the more general uses of the method for mapping the whole fungal genome and for the detection of dispersed repetitive sequences will be discussed.

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51. The molecular biology of *Penicillium chrysogenum*

P. Ford, G. Saunders and G. Holt

School of Biotechnology, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS.

Our investigations into the molecular biology of *Penicillium chrysogenum* have involved the construction of gene libraries and the cloning of fungal genes by complementation. In addition, analysis of mitochondrial DNA from a range of wild type isolates, and the isolation of autonomously replicating sequences has been undertaken. Using the restriction enzymes BamHI and HindIII, chromosomal DNA from *P. chrysogenum* NRRL1951 and pBR328, two gene banks have been constructed. Plasmid pools from recombinants have been used to isolate genes from *P. chrysogenum* able to complement the *E. coli* mutations *proA*, *leuB* and *trpC* (Holt *et al.*, Proc. 16th Workshop Conference Hoescht, Verlag Chemie, in press).

Restriction enzyme analysis of the mitochondrial DNA from 20 wild type isolates of *P. chrysogenum* has revealed that the fragmentation pattern of the mtDNA from these strains is generally the same. However, three strains did show polymorphism in their restriction enzyme sites. We have calculated the molecular weight of mitochondrial DNA from *P. chrysogenum* NRRL1951, isolated by caesium chloride density gradient centrifugation, to be 28·1 Kb. The molecular weights of the mitochondrial genomes from the three other wild type isolates are 26·4, 26·1 and 21·5 Kb. To date, the latter figure is the smallest mitochondrial genome size reported for a filamentous fungus (Sederoff, *Adv. Genet.*, *22*, 2, 1984; Minuth *et al.*, *Curr. Genet.*, *5*, 227–1982).

Seventy-three per cent of the mitochondrial DNA from *P. chrysogenum* NRRL1951 has been cloned into the yeast integrative vector pMa700. Recombinant plasmids containing the 8·5, 7·1, 2·6 and 2·4 Kb EcoRI generated fragments have been used to transform *Saccharomyces cerevisiae* MD40/4C at high frequency. pMa700 usually transforms at a frequency of 1–10 transformants per μg of plasmid DNA (Montiel *et al.*, *Nucl. Acid Res.*, *12*, 1049, 1984) whereas the recombinant plasmids gave a range of frequencies from $1{\cdot}1 \times 10^3$ to $3{\cdot}7 \times 10^3$ transformants per μg plasmid DNA and the yeast replicating vector pMa3a transformed MD40/4C at a frequency of $2{\cdot}7 \times 10^3$ transformants per μg plasmid DNA. This strongly suggests that all four EcoRI mitochondrial DNA fragments contain a sequence which is able to confer on pMa700 the ability to replicate autonomously in *S. cerevisiae*.

52. Analysis of mitotic recombination with an excision-resynthesis model and implications for meiotic recombination

P. Unrau and J. R. Johnson

A.E.C.L. Health Sciences Division, Chalk River, Ontario, Canada K0J 1J0.

The effects on gene conversion of specific mutations in a known DNA sequence have been studied by Moore and Sherman (*Genetics*, *70*, 397, 1975; *Genetics*, *81*, 1, 1977). We have analysed their data in conjunction with an excision-resynthesis-driven heteroduplex model based on that of Resnick (*J. theor. Biol.*, *59*, 97, 1976). On our model the probability of gene conversion is a function of the probability, 'H', that the repair heteroduplex reaches at least one mutant allele, the probability 'S', that the repair heteroduplex stops at a mismatch and the

probability 'P', that the mismatch is resolved in favour of the wild-type allele. This model can be used to explain the observations of Moore and Sherman. For example, the observation that crosses involving alleles less than 5 base pairs apart exhibit low induced recombination rates as compared to crosses 5 or more base pairs apart can be explained if the presence of the second mismatch within 4 base pairs results in a low value of 'S' (this has implications for the heteroduplex junction). Crosses between frameshift deletions and base substitutions give very high recombination (both induced and meiotic) and are explicable if 'S' and 'P' are near unity at the frameshift deletion. Meiosis exhibits map expansion in crosses between base substitutions but induced recombination does not. This is expected if the probability 'H' independent of separation for induced but not meiotic gene conversion (implying preferred recombination initiation sites in meiosis). That a single model can be used to explain the similarities and differences in meiotic and mitotic recombination rates supports an argument in favour of the underlying unity of two processes.