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CELL WALL COMPOSITION AND LYTIC ENZYME ACTIVITY ASSOCIATED WITH DIKARYON MORPHOGENESIS IN *COPRINUS*

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The incompatibility factors in tetrapolar basidiomycetes each control part of a well characterised morphogenetic sequence of events which lead to dikaryon formation from two compatible monokaryons. Functions assigned to the *B* factor are septal dissolution in the monokaryon and clamp cell fusion in the dikaryon, both of which allow nuclear migration and both involve lytic enzyme activity. Mutation in the *B* factor leads to loss of incompatibility control and can also make *B* functions constitutive. Such mutations are useful for biochemical analysis of *B* functions. *B* mutants of *Coprinus* have recently been obtained using a specially developed technique which will be described.

Contrary to the situation found in the only other tetrapolar species to be examined, *Schizophyllum commune* [Wessels and Niederpruen, *J. Bacteriol. 94*: 1594 (1976); Wessels, *J. Bacteriol. 98*: 697 (1969)], there is no marked decrease in glucan content of the cell wall in *B* mutants, nor the associated increase in a glucan specific lytic enzyme. Such an enzyme is, however, implicated in *B* functions and effects *in vitro* dissolution of septa. Possible reasons for the differences in the two fungi are discussed.

"*Super*"—A GENETIC CONTROL FACTOR GIVING VERY HIGH GENE CONVERSION FREQUENCIES IN *ASCOBOLUS IMMERSUS*

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The "*Super*" monogenic control factor greatly enhances gene conversion frequencies for two *white* (*w*) spore colour mutations at locus I in Pasadena strains of *Ascobolus immersus*. Total conversion frequencies at these sites were about 25 per cent in $+ \times w$ crosses heterozygous for "*Super*", compared with only about 0·88 per cent for the same kind of cross without "*Super*" but homozygous for control factor "91". Associated features with the enhancement of total conversion frequency by "*Super*" were increased postmeiotic segregation and increased conversion to + rather than to *w*. Unlike the "*P*", "*K*" and "*91*" control factors which affect the gene conversion frequency at various sites in locus I and are closely linked to that locus (Lamb and Helmi, *Genet. Res.*, in press), "Super" is unlinked to it, perhaps non-synnemal.

The origin of the new control factor "*Super*", its action in *cis* and *trans* positions, dominance, specificity and interactions with the other control factors that affect the conversion properties at locus I were studied. Such high conversion frequencies and disparity in frequencies of conversion to wild-type and to mutant could affect allele frequencies in polymorphic populations. Conversion frequencies as high as 25 per cent show that hybrid DNA formation at a given site may be very frequent and not just a random rare event as is often supposed.

LACTAM UTILISATION IN *ASPERGILLUS NIDULANS*: EVIDENCE FOR A FOURTH GENE UNDER THE CONTROL OF THE INTEGRATOR GENE *intA*

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The *intA* gene of *Aspergillus nidulans* is a positive acting regulatory gene which can be formally described as an integrator gene on the Britten and Davidson model for gene regulation

[Arst, *Nature 262*: 231 (1976)]. The *intA* product mediates the induction by β-alanine, γ-amino-*n*-butyrate (GABA) and certain other ω-amino acids of the syntheses of at least three putative structural gene products: acetamidase, GABA permease, and a transaminase for GABA, β-alanine and several other ω-amino acids. The rationale for integrated expression of these three activities is unclear, but identification of further structural genes under *intA* control should provide additional clues as to why *intA* exists.

lamA, in linkage group VIII, is probably a fourth gene under *intA* control. Mutations in *lamA* prevent conversion of exogenous 2-pyrrolidone (γ-butyrolactam) to GABA and the (probably analogous) utilisation of 2-piperidone (δ-valerolactam). *lamA* probably specifies a lactamase, but a role in lactam uptake cannot be ruled out.

THE EFFECT OF STARVATION ON THE SYNTHESIS AND MAINTENANCE OF P^1 dsRNA IN *SACCHAROMYCES CEREVISIAE*

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The yeast virus-like particle (VLP) neither lyses nor infects its host cell and therefore can only be propagated through cellular growth and division. This constraint requires that VLP replication is coordinated with host cell metabolism. We have investigated the effect of amino acid and nitrogen starvation on the synthesis of P^1 double-stranded ribonucleic acid (P^1 dsRNA), the VLP genome.

When protein synthesis is prevented by starvation for a required amino acid or by addition of cycloheximide, the rate of P^1 dsRNA synthesis is reduced markedly. During nitrogen starvation the synthesis of P^1 dsRNA continues but is accompanied by the degradation of pre-existing molecules. This degradation appears to require the induction of new enzymes and it is likely that breakdown products are used to enable the cell to complete its division cycle. However, all of the copies of the VLP genome are not degraded in this process, some are conserved and can replenish the amount of P^1 dsRNA on return to growth conditions. Hence the pattern of VLP replication is well-adapted to its dependence on the survival of the host cell.

NUCLEAR MUTATIONS IN *SACCHAROMYCES CEREVISIAE* WHICH AFFECT THE SPONTANEOUS MUTATION FREQUENCY IN MITOCHONDRIAL DNA

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Fourteen mutants have been identified in which the frequency of spontaneous mutations in mitochondrial DNA is increased. As well as increasing the frequency of mutations to resistance to erythromycin, oligomycin and spiramycin, all the mutants also show changes in the frequency of spontaneous petite induction. None of the mutants has any effect on the frequency of spontaneous nuclear mutations. Nine of the mutants are in one complementation group and five are in another. The phenotype of both groups is caused by a single nuclear mutation. A fifteenth mutant defines a separate complementation group and increases the mutation frequency on both the mitochondrial and nuclear genomes.

OBSERVATIONS ON MITOCHONDRIAL DNA SYNTHESIS IN A YEAST MUTANT DEFECTIVE IN DNA LIGASE

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The temperature sensitive cell cycle of mutant *Saccharomyces cerevisiae, cdc9*, has been shown to have a lesion in a DNA ligase involved in replication of nuclear DNA. When growing cells are shifted from the permissive (25°C) to the non-permissive (37°C) conditions, synthesis of nuclear DNA continues for about a generation. Newly synthesised DNA single strands accumulate as small pieces comparable in size with Okazaki fragments, while the parental strands remain essentially unchanged in size (Johnston and Nasmyth, 1978).

Extending observations on this mutant to the mitochondrial system, we have now found that mitochondrial DNA synthesis also continues, at least for a limited period, after the switch to 37°C. However in this case there is no accumulation of fragments like those observed with nuclear DNA and little difference has been seen between the parental and daughter single strands. This suggests either that the replication of mitochondrial DNA does not require the gene product specified by *cdc9* or that the replication mechanism of mitochondrial DNA differs fundamentally from that of nuclear DNA. Experiments aimed at resolving this problem are in progress.

THE STUDY OF MITOCHONDRIAL INHERITANCE BY PROTOPLAST FUSION

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In yeasts, mitochondrial genes are characterised by non-Mendelian inheritance, mitotic segregation, and deletion through treatment with ethidium bromide. However, the study of mitochondrial inheritance in the *petite*-negative yeast, *Kluyveromyces lactis*, is complicated by unstable diploidy, and sterility of certain respiratory-deficient mutants. We have isolated stable hybrids from a heterothallic strain of *K. lactis* by protoplast fusion between auxotrophic mutants of identical mating-type. These prototrophic hybrid cells have been shown to be of greater size and to carry approximately twice the DNA content of haploid parentals. Nuclear staining of hybrid cells reveals only one nucleus. Thus, it appears that these fusion products are diploid. Two erythromycin-resistant mutants of *K. lactis* have been shown to exhibit Mendelian and non-Mendelain inheritance respectively. Heteroplasmic hybrid formation (ery^R/ery^S) by protoplast fusion permits mitotic segration analysis. By treatment with ethidium bromide and subsequent protoplast fusion with respiratory-competent cells (" transfusion ") it has been possible to demonstrate chromosomal and mitochondrial inheritance of erythromycin-resistance in *K. lactis* by the usual criteria.

ARE CONSISTENT CHROMOSOME ABNORMALITIES IN HUMAN MALIGNANCY RELATED TO TRANSPOSABLE ELEMENTS?

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The occurrence of consistent translocations is one of the most remarkable observations that has resulted from the chromosome analysis of human haematologic malignancies. There are four such consistent translocations, each one reasonably specifically associated with a particular malignancy. They include t(9q+; 22q−) in chronic myelogenous leukaemia, t(8q−; 21q+) in acute myeloblastic leukaemia, t(15+; 17q−) in acute promyelocytic leukaemia, and t(8q−; 14q+) in Burkitt lymphoma.

It is appropriate therefore, to ask how and why these translocations occur in particular malignacies. These rearrangements may result from chromosomal proximity or DNA homology. It has been shown however, that transposable elements can cause large-scale rearrangements of adjacent DNA sequences. The properties of controlling elements are (1) change in location within the DNA, (2) transfer of adjacent DNA in this change, and (3) alteration of the normal mechanism for genetic regulation, depending on the site and orientation of the inserted sequences. These properties, plus a selective system to remove changes that do not have a proliferative advantage in haematologic cells, are just those required to explain consistent translocations occurring as somatic mutations.

GENETIC CONTROL OF SEVERE PRE-ECLAMPSIA

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Pre-eclampsia (toxaemia, gestosis) is the name given to hypertension of pregnancy. Severe pre-eclampsia is also usually characterised by oedema and proteinuria, and is probably a distinct entity from the milder forms of pre-eclampsia. It occurs much more frequently

in first than in later pregnancies. Several bodies of data on its familial occurrence have been published. The data consist of information on the frequency of occurrence of the condition among the mothers, daughters and sisters of normal, severely pre-eclamptic and eclamptic index cases. No previous attempt seems to have been made to establish whether these data are compatible with Mendelian modes of inheritance. The expected frequencies amongst the various classes of relatives have been derived on the hypotheses of simple dominant and simple recessive inheritance. Maximum likelihood analysis suggests that severe pre-eclampsia may be inherited as a simple recessive trait. The data are not sufficient to decide whether it is the genotype of the mother or of her foetus which predisposes towards severe pre-eclampsia. An association between parental HLA types and severe pre-eclampsia recently reported by Redman *et al.* (*Lancet ii*, 397-399, 1978) suggests that the putative gene may be linked to this locus.

AMNIOCENTESIS IN THE WEST MIDLANDS—AN AUDIT OF 1000 CASES

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In the West Midlands, 1000 "at risk" pregnancies of known outcome have been audited. About half were for potential spina bifida. AFP level was estimated in 982 (98 per cent). Of these 966 mothers could be reassured that the baby would be unlikely to have an open neural tube defect; 16 abnormal fetuses were terminated or died spontaneously. Chromsome studies were completed in 727 (75 per cent) of the pregnancies, 710 mothers were reassured and 16 pregnancies terminated. Of these, 10 had an abnormal karyotype, including five mongols. Six male fetuses were terminated due to a high chance of carrying an X-linked disease. Three abnormal babies survived. One was a covered encephalocoele with a normal AFP value, one mongol resulted from a pregnancy whose amniotic fluid did not have chromosomes studied and one ambiguous chromosome result preceded the birth of a baby with the signs of Turner's syndrome.

As this study is based on the West Midlands audit, it relates exclusively to data acquired passively from hospital records and capable of being summarised within the confines of a punched card. In view of this, and of its confidentiality, only broad statistical issues will be discussed. The audit is known to be almost complete, excepting failures to acquire fluid. Some potential patients rejected on grounds of twinning are also unrecorded.

RADIOSENSITIVITY IN HUNTINGTON'S CHOREA CELL STRAINS: A POSSIBLE PRE-CLINICAL DIAGNOSIS

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Huntington's chorea (HC) is a relatively common (1 in 10,000 in N. Europe) autosomal dominant abnormality. The mean age of onset is 35 years with a range of from 15-65 years, as a consequence afflicted individuals will usually have produced offspring before showing symptoms. The identification of individuals at risk then becomes a problem of counselling since half the children of an affected parent will be expected to develop the disease.

Fibroblast cell strains were obtained from the Human Genetic Mutant Cell Repository, Camden, N.J. Four cell strains from diagnosed cases proved to be more sensitive to γ-irradiation than cultures from normal individuals (Do, HC = 70 rad, normal = 125 rad). Since cell strains from three " at risk " individuals also proved sensitive this test provides the possibility of a pre-clinical diagnosis. Although the association between HC and sensitivity to γ irradiation may be operationally indirect, it does raise the possiblity that DNA repair may be implicated in the maintenance of neurological function.

TRUE HERMAPHRODITISM, XX MALES AND THE H-Y ANTIGEN

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The H-Y antigen, a cell surface component present in males of all mammalian species tested, is expressed also in absence of a Y chromosome but it is closely correlated with the

presence of testicular tissue. Two sibs, one female and the other of male gender labelled as true hermaphrodite and male XX, respectively are both H-Y positive. This observation and other evidence support a multiple genetical control of H-Y expression with the possible involvement of loci on an autosome, the X- and the Y chromosome. This hypothesis, due to Ulrich Wolf, is discussed.

COMPLEMENTATION ANALYSIS OF THE *VESTIGIAL* MUTATIONS OF *DROSOPHILA MELANOGASTER*

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Amongst the *vestigial* mutations are dominant and recessive point mutations, chromosomal deletions and inversions which affect both embryonic and adult phenotypes. The *vestigial* locus maps on the second chromosome at 67·0. The *vestigial* mutations affect wing size. The defects of the *vestigial* alleles range from vestiges of wings to wings with nicks. Some of the *vestigial* alleles when homozygous are female steriles or zygotic embryonic lethals. Since there is a large range of abnormalities which are expressed by a series of overlapping deficiences covering the *vestigial* locus, we did a complementation analysis using all the *vestigial* alleles to see which particular areas of the chromosome caused the various abnormalities such as female sterility or embryonic lethality.

Thus a chromosomal map can be drawn showing which bands in this region of the second chromosome when deleted lead to embryonic lethality, female sterility and wing abnormalities.

THE GENETICS OF FLOWERING TIME DIFFERENCES IN *ANTIRRHINUM MAJUS* L.

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Four inbred lines of *Antirrhinum majus* which differ in the time of flower formation when grown at 25°C have been studied. Of these lines, three (105, 106 and Sippe 50) produce their first flower buds several days before the fourth line (101). In the crosses between 101 and the other lines, the F_1 plants flower at the same time as the early parents. The segregation of budding time differences has been investigated in the F_2 and backcross generations for crosses between 101 and the early budding lines. In the cross 101 × 105, lines formed by recurrent back-crossing to the parental lines have also been studied. The genetic variation appears to be wholly polygenic. The effects of some environmental factors on flowering in the lines were studied. Of these, temperature has the greatest effect on the expression of the flowering differences and also shows interactions with the genetic variation.

CYCLIC CHANGES AT THE *pal* LOCUS IN *ANTIRRHINUM MAJUS*

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In 1976, Sastry (*Heredity, 36*, 315) described an unusual *pal* allele ($pal^{rec\text{-}low}$) which, unlike the standard unstable pal^{rec} [see Fincham (1973), *Genetics, 73* (Supp.): 195], is colourless (except for one or two rare late mutant spots) in the original homozygous condition. However, when $pal^{rec\text{-}low}$ is made heterozygous with a recessive pal^{tub} (stably colourless) different levels of instability are evoked which show considerable heritability. In a proportion of heterozygous individuals several shifts take place from a relatively colourless, stable condition to a highly unstable state. This shifting process also shows a high degree of heritability in early generations, but gradually disappears, resulting in plants with different but uniform levels of instability. We will present evidence to show that (a) the repression of instability in the original homozygous $pal^{rec\text{-}low}$ plants and the cyclic shifts in the heterozygotes are controlled by an independently located factor (factors?) and (b) the regulatory relationship is very specific since only $pal^{rec\text{-}low}$ can respond to the controlling action of this

factor. These results are discussed in relation to those on cyclic activation and inactivation of controlling elements in maize [see McClintock (1965) *Brookhaven Symp., 18.*, 162].

STRUCTURE AND FUNCTION OF THE CHLOROPLAST GENOME

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Chloroplasts of higher plants contain multiple copies of a covalently-closed naked circular DNA molecule with a molecular weight. depending upon the species, of 85 to 95×10^6. Denaturation mapping, renaturation kinetics and restriction endonuclease analysis show that the majority of the circular molecules in each circle is at least 80 per cent. unique. In *Euglena* it is established that the nuclear DNA does not contain even a single copy of the chloroplast genome, but the position in higher plants is still obscure. In some, but not all, higher plants, each circle of chloroplast DNA contains two copies of an identical sequence arranged in an inverted orientation; these inverted repeats contain the genes for chloroplast ribosomal RNAs. The 5S, 16S and 23S RNA genes are transcribed as a single unit to give an RNA precursor of mol. wt. $2{\cdot}7 \times 10^6$ which is synthesised by isolated chloroplasts. Other genes present in chloroplast DNA include those for chloroplast tRNAs, the large sub-unit of ribulose bisphosphate carboxylase (Fraction I protein), and several thylakoid components of the ATP-synthase complex. Many cloned fragments of chloroplast DNA are now available, and gene mapping studies are in progress. Hybridisation studies suggest that most of the chloroplast genome is transcribed to give RNA populations of different abundance classes. Studies of protein synthesis by isolated chloroplasts and by reconstituted transcription-translation systems suggest that the chloroplast genome encodes at least 100 polypeptides, but most of these are unidentified. The chloroplast genome functions in co-operation with the nuclear genome, since many chloroplast proteins are encoded in the nucleus and synthesised in the cytoplasm. Recent work on this co-operation is discussed with regard to the synthesis of Fraction I protein.

MITOCHONDRIAL BIOGENESIS IN *PARAMECIUM*

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A study of mitochondrial biogenesis in *Paramecium* has been directed towards examining three main topics, namely:
1. The rules governing the segration of mitochondrial genomes in mixed populations of genetically marked mitochondria; 2. The structure and function of the mitochondrial genome, and 3. The interaction of nuclear and mitochondrial genes involved in mitochondrial biogenesis.

Evidence is presented which demonstrates that mutants' resistance to various antobiotics is mitochondrially determined. Experiments using cells containing two genetically marked populations of mitochondria show that recombination between mitochondrial genes cannot be detected and that mitochondrial genomes segregate independently according to their relative selective advantages within the cell. The function of mitochondrial DNA has been examined using biochemical analysis of mutants, DNA-RNA hybridisation and by use of species variation in mitochondrial proteins. The results give information on the genetic determination of mitochondrial ribosomes, soluble mitochondrial enzymes, membrane proteins and the ATPase complex. Nucleocytoplasmic interactions have been investigated by studies of incompatibility of various nuclear/mitochondrial combinations using both mutants and various different species combinations produced by micro-injection.

MITOCHONDRIAL GENETICS OF FILAMENTOUS ASCOMYCETES

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While advances in the mitochondrial genetics of *Saccharomyces cerevisiae* are far greater than for any other organism, studies on *Neurospora crassa* and *Aspergillus nidulans* have led to

some interesting insights into the evolution of mitochondria. Although these two organisms differ considerably in the size of their respective mitochondrial genomes (MW Aspergillus mito DNA, 22×10^6; Neurospora 40×10^6), a close similarity is seen in the structure and synthesis of mitchondrial ATP synthetase in the two organisms. In *A. nidulans*, both nuclearly and extranuclearly-inherited mutations confer oligomycin-resistance on the activity of the enzyme complex (Rowlands and Turner, 1977, *Molec. gen. Genet.*, *154*, 311), in contrast to yeast, where oligomycin-resistance mutations affecting the enzyme have been observed only in the mitochondrial genome. To date, only nuclear oligomycin-resistant mutants have been isolated in *Neurospora crassa*, and as in Aspergillus, these seem to lie within a single gene. Recent studies on the product of this nuclear gene in *N. crassa* (Sebald, Sebald-Althaus and Wachter, 1977, in " Genetics and Biogenesis of Mitochondria ", Bandlow *et al.* (eds.)), have revealed a striking difference in the biosynthesis of the ATP synthetase complex of this organism from that of yeast, and *A. nidulans* is apparently similar to Neurospora in this respect.

MOSAIC ORGANISATION AND EXPRESSION OF THE MITOCHONDRIAL DNA REGION CONTROLLING CYTOCHROME b AND CYTOCHROME OXIDASE

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Mitochondria have an independent genome the function of which is to provide information and machinery in order to synthesise polypeptides of the inner membrane. These polypeptides are few in number (less than a dozen) but critical in importance for cellular respiration and oxidative phosphorylation. In the last few years the general rules of genetics, the overall organisation of mitochondrial DNA, its main functions and mechanisms of organelle biogenesis have been studied (for recent Symposia see 1-4).

Yeast mitochondrial DNA is 75,000 bp long. A region situated in the sector S3 of the genetic map (see ref. 5) and between the 14,000 bp and 24,000 bp positions of the physical map has particularly interesting properties. This region, as shown by crosses and restriction analysis of cloned DNA segments, displays a mosaic organisation of genetic information, *i.e.* coding and regulatory sequence controlling cytochrome *b* (of the cytochrome *c* reductase complex) and subunit I of the cytochrome *c* oxidase complex) are interspersed (6). Three distant genetic loci represent cytochrome *b* exons. Deficient mutants (7,8,9) and drug resistant mutants (10) are located in them. They modify the structure of a single cytochrome *b* polypeptide chain (11) and constitute a single unit of complementation (6). Within this cistron other mutations controlling cytochrome reductase and oxidase are located in introns. They constitute distinct units of complementation, present characteristic features of mitochondrially translated new polypeptides and a specific pattern of regulation.

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FOUR-POINT MITOCHONDRIAL CROSSES IN *ASPERGILLUS* NIDULANS

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The markers used were (*camA112*)- chloramphenicol resistance (Gunatilleke *et al.*, *Molec. gen. Genet.*, *137*, 269, 1975); (*oliA1*)- oligomycin resistance (Rowlands and Turner, *Molec. gen. Genet.*, *126*, 201, 1973); (*cs67*)- cold-sensitivity (Waldron and Roberts, *J. gen. Microbiol.*, *78*, 379, 1973) and (*sumD16*), a partial suppressor of (*cs67*). Strains carrying (*sumD16*) were phenotypically indistinguishable from the wild-type. Heterokaryon sector analysis, based on the observation that heterokaryons produce sectors which are homogenous for the mitochondrial genome and the assumption that these represent independent segregational events, showed that (*camA112*), (*cs67*) and (*sumD16*) were linked.

Eight different four-point mitochondrial crosses were conducted in heterokaryons with (*camA112*) arranged in every possible permutation. Conidia from young heterokaryons were collected and (*camA112 oliA1*) double recombinants selected at 37°C. These were scored for their growth phenotype at 20°C. Although only three phenotypes, representing four genotypes, were identifiable, on the assumption that the mitochondrial map is circular, a combined analysis of the eight crosses suggested that (*camA112*), (*cs67*) and (*sumD16*) were linked and mapped in the order given. Although (*cs67*) and (*sumD16*) recombined quite freely, it cannot be stated whether they map in different genes.

A SPLIT GENE IN THE MITOCHONDRIAL DNA OF *NEUROSPORA CRASSA*

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Workers in several laboratories are currently engaged in studies aimed at locating specific coding regions on physical maps of mitchondrial DNA. A particularly successful technique now in use is the hybridisation of specific RNA molecules to DNA fragments immobilised on nitrocellulose filters by the method of Southern (*J. molec. Biol. 98*: 503-517, 1957). In the light of recent discoveries regarding the splicing of genes in eukaryotes (see for example Gilbert, *Nature*, *271*, 501, 1978), it is now apparent that it may be dangerous to assume that restriction fragments hybridising a single RNA species are contiguous. Terpstra *et al.* (*Biochem. Biophys, Acta*, *478*, 145-146, 1977) located mitochondrial ribosomal RNA from *Neurospora crassa* on specific DNA fragments and used their hybridisation data in the refinement of their restriction maps. Our hybridisation data confirms that of Terpstra *et al.*, but construction of restriction maps without recourse to such data clearly shows that fragments hybridising the ribosomal large subunit RNA are not contiguous, indicating that the gene is split. Preliminary results suggest the existence of a split in the gene coding for the mitochondrial ribosomal large subunit RNA of *Aspergillus nidulans*, so that, together with the report of split mitochondrial ribosomal RNA genes in yeast (Borst *et al.*, *Nature*, in the press), a general phenomenon may be indicated.

PRELIMINARY ANTIMITOCHONDRIAL ACTIVITY OF CHEMICAL CARCINOGENS CAUSES TRANSFORMATIONAL TYPE CHANGES AT THE YEAST CELL SURFACE

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Sixteen chemical carcinogens when tested were found to affect the mitochondrial biogenesis selectively in *S. cerevisiae*, potency varying with the strain and the carcinogen. Inhibitory effects were detected as depression of mitochondrial protein synthesis and induction of the petite mutation, presumed to be due respectively to transcription and replication aberrations of mitochondrial DNA. The latter produce irreversible, and the former reversible, respiratory deficiency but also cause changes at the cell surface. These were detected as (1) change in agglutinability of whole cells with concanavalin A, (2) inability to take up and utilise certain sugars other than glucose, an effect which can be overcome by permeabilising

cells with DMSO (Mahler and Wilkie, *Plasmid 1*: 117 1978), (3) reversion of flocculation, (4) changes in cellular tolerance tochloripramine and cycloheximide, (5) alteration in the capacity to secrete amylase and protease. Some function of the mitochondrial inner membrane system other than the production of ATP seems to be involved in the control of cell surface characteristics.

ISOLATION OF AN ANTIMYCIN A RESISTANT HUMAN CELL LINE

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A cell line resistant to the electron transport inhibitor antimycin A has been isolated following the mutagenesis of HeLa with N-methyl-N-nitro-N-nitrosoguanidine. The variant line has been cloned twice in 15 μM antimycin A, a concentration ten times greater than that which kills all parental cells. The growth rate and oxygen uptake of the variant cell line are apparently unaffected by concentrations of the drug up to 15 μM. However the activity of succinate/cytochrome *c* reductase in membrane preparations from the variant was found to be similar to that of preparations from HeLa and showed full sensitivity to antimycin. The properties of the variant were stably propagated in the absence of selection over a period of two and a half months. One distinguishing feature of the variant cell line is the apparent increased production of a mitochondrially synthesised protein of molecular weight 29,000 Daltons. Experiments are in progress to test the hypothesis that the resistance is transmitted cytoplasmically.

THE RANDOM SORTING-OUT OF PLASTIDS HYPOTHESIS REBUFFED

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The hypothesis that normal and mutant plastids, introduced into biparental zygotes at fertilisation, sort-out directly into the embryo cells at random is untenable for *Pelargonium*. Instead, the patterns of segregation into green, variegated and white embryos after G × W plastid crosses, indicate that quite a different model is needed. It is suggested that between fertilisation and the maturation of the zygotes there is a radical alteration in the plastid content. Initially, most plastids are restricted until only one or, less often, two plastids remain. The surviving plastid then replicates to produce many new copies of itself or, when two unlike plastids survive, one is usually selected to replicate in preference to the other. The probability of survival and replication for a normal mutant plastid is controlled largely by the nuclear genotype of the female parent, and to a lesser extent by inherent differences between the plastids themselves.

SYNTHESIS OF CYTOCHROME *f* BY ISOLATED PEA CHLOROPLASTS

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Cytochrome *f* can be isolated from the membranes of pea chloroplasts by extraction with a mixture of ethyl acetate, ethanol and ammonia, followed by precipitation with a specific antiserum. After separation from the immunoglobulins by gel electrophoresis in sodium dodecylsulphate, the cytochrome *f* polypeptide can be identified by its intrinsic fluorescence in ultraviolet light. If, before extraction, the chloroplasts are incubated with ^{35}S-methionine or ^{3}H-leucine, cytochrome *f* is found to be radioactive. The incorporation of label into cytochrome *f* continues in the presence of cycloheximide and ribonuclease but does not occur in the dark, or in the presence of D-chloramphenicol. We conclude that the observed synthesis of cytochrome *f* is not due to contamination with cytoplasmic polysomes or bacteria but is occurring in intact chloroplasts using energy derived from the light reactions of photosynthesis.

CONTROL INTEGRATING GROWTH RATE AND THE INITIATION OF MITOSIS IN FISSION YEAST

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Allosuppressors have been isolated in the fission yeast *Schizosaccharomyces pombe*. These allosuppressors restore activity to a nonsense suppressor tRNA which has been inactivated by second site mutations within the gene coding for the tRNA. One allele of one allosuppressor has an interesting pleiotropic effect on the initiation of mitosis. At 25°C mitosis is initiated at a much larger cell mass than normal, whilst at 35°C mitosis cannot be initiated at all. Thus this mutational alteration in the allosupressor gene has two effects:

(1) Restoration of activity of an inactive nonsense suppressor tRNA, presumably either by affecting the processing or synthesis of the tRNA, or by affecting the ribosome such that the tRNA can now function.

(2) Conditional inhibition of the initiation of mitosis.

Mitosis can also be temporally inhibited if cells are transferred to conditions supporting a fast rate of growth (*Experimental Cell Research*, *107*, 377, 1977), suggesting that cells have some mechanism for monitoring growth rate and relating this information to the initiation of mitosis. Since the signal monitoring growth rate could be generated at the ribosome, the allosuppressor gene may be involved in this control coordinating growth rate and mitosis.