THE GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers presented at the HUNDRED AND SEVENTY-FIRST MEETING of the Society held on 4th, 5th and 6th April 1973 in the Biological Laboratory, UNIVERSITY OF KENT, CANTERBURY.

THE CURRENT STATE OF *STREPTOMYCES* GENETICS

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Genetic recombination occurs in most strains of *Streptomyces* in which it has been sought. In those studies in which the analysis would have revealed it the hallmark of conjugation is evident: the transfer of long groups of linked genes. There is as yet no unambiguous evidence of transduction or transformation, but the recent isolation of temperate phages for *S. coelicolor* A3(2) may soon lead to a useful transduction system.

In *Streptomyces coelicolor*, *S. rimosis*, *S. bikiniensis* and *S. glaucescens* a circular linkage map has been constructed. There is suggestive evidence of a conserved linkage arrangement in the genus; *S. coelicolor* and *S. rimosus* have very similar linkage maps.

A plasmid, SCP1, plays an important role in gene transfer in *S. coelicolor* A3(2). The plasmid is autonomous in IF strains, absent from UF strains and integrated at a particular position on the chromosome in NF (donor) strains. In other donor strains (see the paper by A. Vivian at this meeting) the plasmid interacts with other regions of the chromosome, sometimes transiently. Even in the absence of SCP1, that is in UF × UF crosses, considerable recombination occurs. SCP1 has been found to be transferred to at least one other wild-type strain.

The known features of the genetics of *Streptomyces* suggest several rational approaches to strain improvement, such as the selective mutagenesis of particular chromosomal regions, the construction of recombinants with defined regions of two parental genomes, and the transfer of characters between different wild-types on substituted plasmids.

THE GENETICS OF PENICILLIN PRODUCTION IN *ASPERGILLUS NIDULANS*

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An understanding of the hereditary control of the overproduction of important secondary metabolites like antibiotics could suggest ways of manipulating the genetic material leading not only to raised antibiotic yields but also perhaps to the isolation of altered and improved chemotherapeutic agents. When *Penicillium chrysogenum* began to be used for the commercial production of penicillin, a fungal survey indicated that strains of *Aspergillus nidulans* were amongst those exuding penicillin-like substances. We confirmed that strains of this fungus elaborated a metabolite indistinguishable from penicillin and showed that production was under nuclear control (G. Holt and K. D. Macdonald, *Antonie van Leeuwenhoek, 34*, 409, 1968). Preliminary hybridisation experiments between different wild type isolates producing the same penicillin titre indicated that they carried different genes responsible for increasing yields (G. Holt and K. D. Macdonald, *Nature, 219*: 636, 1968). A programme was then initiated to produce single mutants with raised penicillin yields from derivatives

of the Glasgow wild type strain NRRL 194. Several of these have now been isolated after ultra-violet light treatment and some have been assigned to specific linkage groups by haploidisation analysis. Experiments are also in progress to position these mutations more accurately by cleistothecial analysis.

THE GENETICS OF PENICILLIN PRODUCTION IN *PENICILLIUM CHRYSOGENUM*

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Improvement in the penicillin yield of *Penicillium chrysogenum* was achieved initially by mutation and selection. Discovery of the parasexual cycle in *P. chrysogenum* (G. Pontecorvo and G. Sermonti, *J. Gen. Microbiol. 11*, 94, 1954) pointed the way to the planned breeding of strains. Although industry has apparently been slow to exploit this discovery, recent published work suggests that the situation is now changing.

The parasexual cycle, with its two main components of mitotic crossing over and haploidisation, is the only gene exchange method so far demonstrated in *P. chrysogenum.* Since both processes are entirely vegetative there is a strong possibility of selection acting in favour of faster growing segregants and against slow growing types. Methods for solving this problem will be discussed together with methods for overcoming the main barrier to effective breeding, namely the low frequency of viable recombinant recovery.

In addition, the relative merits of fundamental and empirical approaches to the breeding of strains will be discussed in the light of recent discoveries not only with *P. chrysogenum* but with other fungi.

CHANGES IN THE METABOLISM OF MUTANTS DURING STRAIN IMPROVEMENT PROGRAMMES

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It is generally thought that, with antibiotic producing cultures, the stepwise improvements which occur during mutation and selection are due to increases in the antibiotic forming enzymes and hence to increases in the specific rate of production per gram of cells. Investigation of information in the literature and data from a series of mutants of *Penicillium patulum*, producing griseofulvin, showed that this occurred in some cases: in others the increased production was due to changes in the general metabolism of the cells or to an improved response to the conditions of culture. These results indicate that many factors can be involved in increased productivity, when secondary metabolism takes precedence over growth.

CONTROL OF EXTRACELLULAR PROTEASE IN *ASPERGILLUS NIDULANS*

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Aspergillus nidulans (*biA1*) synthesises three different neutral or alkaline proteases (α, γ, ε) that may be secreted as extracellular enzymes (not two as previously reported). In addition it synthesises one protease (β) that is never secreted. Synthesis of all four proteases is repressed during growth in minimal medium (50 mM-ammonium and 50 mM-glucose) and is derepressed by transfer to and maintenance in ammonium-free or glucose-free media. With protein as sole nitrogen or sole carbon source, simultaneous growth and derepression of protease synthesis and release is obtained, but the results of the transfer experiments show that the presence of substrate protein is not essential for derepression of synthesis or release. The roles of phosphate and

sulphate in protease regulation have not yet been studied, nor have possible acid proteases been investigated.

In cells grown under repressing conditions the intracellular protease (β) is present as two zymogen-like precursors (β1 and β2) which are converted to β with increase in proteolytic activity in stored cell-free mycelial extracts. In depressed cells, protease γ is formed initially as an active precursoe (δ); both δ and γ are secreted but the conversion of δ to γ does not occur in stored, cell-free culture filtrates.

Conversion of β1 and β2 to β is the first detected intracellular response to derepression, occurring about 2 hours after transfer. Synthesis and release of α, γ-δ and ε occurs at about the same time and continues for many hours, γ-δ being the earliest-formed and most active components. In the presence of cycloheximide all effects of derepression, including the intracellular zymogen-like activation of β are blocked.

The effects upon protease regulation of a number of mutations known to affect ammonium repression of other enzymes will be described and the relevance of the results to the regulation of synthesis and secretion will be discussed.

THERMAL INACTIVATION OF YEAST CELLS

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The repair of cell damage induced by heat treatment at 52° and ionising radiations may share common factors, as shown by the cross sensitivity to 50° of X-ray sensitive mutants of both bacteria and the yeast *Sacchoromyces cerevisiae* (Bridges, Ashwood-Smith and Munson, *Biochem. Biophys. Res. Commun.*, *35*, 192-196 (1969); Evans and Parry, *Molec. gen. Genet.*, *118*, 261-271 (1972)).

We have isolated 17 mutants of yeast showing primary sensitivity to heat treatment at 52° (prefixed *hs*). All 17 mutants were appreciably sensitive to EMS and slightly sensitive to X-rays at low doses.

Genetic analysis was performed upon the mutants with limited success. Three mutants failed to mate with wild type cultures whilst the remainder all show low spore viability. In 12 out of 14 of the mutants the *hs* phenotype proved to be partially dominant, the other 2 being recessive.

Heat treatment of wild type diploid cultures of yeast at 52° and 37° during the exponential phase of growth results in increases in both mutation and mitotic gene conversion.

The results indicate that repair mechanisms may have evolved in response to the effects of elevated temperatures, since these show many similarities to those of radiations and chemical mutagens.

THE CORRELATION BETWEEN ds-RNA IN YEAST AND THE "KILLER" CHARACTER

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Analyses of the RNA content of various " killer " and " sensitive " strains of *Saccharomyces cerevisiae* have suggested there may be a correlation between the presence of ds-RNA and the expression of the " killer " character. That such is the case is supported by the results of two types of investigations: firstly, the genetic and biochemical analyses of sensitive mutants induced from killer cells by 5-fluorouracil, and, secondly, the analyses of offspring derived from genetic crosses between killer strains and sensitive strains which differ in both their ds-RNA content and in respect to their nuclear *M*/*m* genotype.

All sensitive mutants so far analysed maintain both their *M* nuclear genotype and the larger ds-RNA molecule but lose their smaller ds-RNA molecule i.e. Killer

$M++ \xrightarrow{5Fu} M+-$. Thus the expression of the killer character is dependent on the presence of the smaller ds-RNA molecule. The retention of the *M* allele in these mutants suggests that the cytoplasmic determinant(s) itself has mutated.

The genetic crosses so far undertaken between killer strains which possess both types of ds-RNA with sensitive strains which differ among themselves in both nuclear genotype and ds-RNA content that all sensitive segregants which have the *m* allele never possess the smaller ds-RNA fraction. Genetic analysis shows that the *m* allele can support the replication of $+-$ but not of $++$.

THE CHARACTERIZATION OF THE YEAST DOUBLE-STRANDED RNA ASSOCIATED WITH THE "KILLER" CHARACTER IN YEAST

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During the course of a search for a nucleic acid fraction associated with the " killer " character in the yeast *Saccharomyces cerevisiae*, E. A. Berry and E. A. Bevan (*Nature*, 1972, *239*, 279) demonstrated the presence in killer strains of a high molecular weight nucleic acid species which they identified as double-stranded RNA (ds-RNA). This molecule has now been further characterized, and a smaller ds-RNA molecule identified which has also been shown to be present in the " killer " strains.

The second smaller molecule, like the larger molecule, has been shown to be ds-RNA on the basis of its nuclease resistance. Further, the melting properties and base ratios of the larger molecule have been shown to be in accord with its double-stranded nature. The molecular weights for the larger and smaller molecules have been found to be 2·5 and $1{\cdot}4 \times 10^6$ respectively. On denaturation and analysis by formamide gel electrophoresis, the higher molecular weight ds-RNA yields a single band of single-stranded RNA suggesting that the molecule is a continuous colinear duplex without nicks.

A survey of a large number of different strains has indicated the existence of three different cell types with regard to their ds-RNA species: those possessing no detectable ds-RNA (designated $--$), those with only the larger ds-RNA molecule ($+-$), and those which possess both types of molecule ($++$). All the $+-$ and $--$ strains have been found to be " sensitive " in phenotype, whilst all " killer " strains are $++$.

Recently two strains have been isolated which are $++$ and " sensitive " but in both cases the lower molecular weight species of ds-RNA has a lower molecular weight than that isolated from " killer " strains.

GENETIC CONTROL IN *AGARICUS BISPORUS*, THE CULTIVATED MUSHROOM

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Agaricus bisporus is the most important fungus to be grown commercially for consumption. The annual value of the U.K. crop is £11·16 m. The last two years have seen considerable advance in our knowledge of the basic genetics of this fungus as a result of work in the U.S.A. (Miller, *Mycologia*, *63*, 630, 1971; Raper, J. R., Raper, C. A. and Miller, *Mycologia*, *64*, 1088, 1972) and in the U.K. (Elliott, *Mush. Sci.*, *8*, 11, 1972).

It has been established that the mushroom is a " secondarily homothallic " species with mating competence almost certainly determined by a bipolar mechanism. An analysis of the nuclear cycle has been completed using complementary auxotrophs isolated from commercial spawns.

Mendelian segregation, heterokaryosis, recombination and the " Buller " phenomenon have been demonstrated. Recent evidence indicates that additional mating

type alleles are present in mushroom spawns, a feature which is typical of bipolar and tetrapolar systems. Characters such as cap colour, number of spores per basidium and aberrant gill structure are being investigated as part of a programme to develop techniques for breeding improved spawns for commerce.

THE INITIAL STAGES OF SEXUAL MORPHOGENESIS IN *CORPRINUS LAGOPUS*

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Although it has been known for some time that the later stages of sexual morphogenesis in *C. lagopus*, a tetrapolar basidiomycete, are regulated by the incompatibility factors the initial stages, hyphal approach, contact and fusion, and nuclear exchange and plasmogamy, have attracted less attention.

It has been found that there is neither restraint nor stimulation of growth between approaching hyphal fronts, though close-range telemorphic effects are difficult to disprove. There is some evidence for temperature-sensitive incompatibility factor control of hyphal fusion. The stimulus to growth found to follow hyphal fusion may be due to exchange of nuclei, but is not necessarily related to their total migration. Growth acceleration occurs in all but non-compatible matings, the size of the effect varying with the type of mating.

Most hyphal fusions involve young cell wall. Differences in rates of wall synthesis have been found between various monokaryons and heterokaryons. Several workers have implicated cell wall synthesising or lysing enzymes in fusion and related phenomena. A detailed study of hydrolytic enzymes has been undertaken and should lead to a better understanding of the operation of the incompatibility factors at this level.

COLD-SENSITIVE MUTANTS OF *ASPERGILLUS NIDULANS*

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Following NMG treatment, 76 mutants of *A. nidulans* have been isolated which grow normally at 37° but not at 20°. Growth tests demonstrate that these mutants (designated *cs*, cold-sensitive) have a range of defects. For example, two are sensitive to deoxycholate, many are osmotic remedials and two (*cs*-13 and *cs*-48) have a specific nutritional requirement for growth at 20° (isoleucine and choline respectively).

Thirty-two mutants were crossed to wild-type and most segregated as single-gene mutations. Genetic analysis shows that mutation to cold-sensitivity can arise in many unlinked loci and affect many functions.

Mutant *cs*-67 exhibits non-Mendelian inheritance in crosses to wild-type, progeny from single hybrid perithecia being either all cold-sensitive or all wild-type. The mutant character is usually lost on passage through forced heterokaryons with wild-type, but occasionally all dissociation products are cold-sensitive.

Cold-sensitive mutants in bacteria are a source of strains defective in ribosome biosynthesis (Tai, Kessler and Ingraham, *J. Bact.*, *97*, 1298, 1969; Gutherie, Nashimoto and Nomura, *P.N.A.S.*, *63*, 384, 1969). Therefore the *Aspergillus cs* mutants were screened for changes in the sedimentation profiles of their ribosomes by sucrose density centrifugation of crude extracts. Four mutants (designated *arp*) produce profiles with an altered ratio of ribosomal subunits after incubation of mycelium at 20°. They produce wild-type profiles during growth at 37°.

Amongst the *arp* mutants, one has increased sensitivity to actidione and two have increased resistance to the drug. However, none of 8 spontaneous mutants selected for actidione-resistance and simultaneous cold-sensitivity produces an altered ribosome profile.

VEGETATIVE DEATH: A MITOCHONDRIAL MUTATION IN *ASPERGILLUS AMSTELODAMI*

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Vegetative death (Vgd) arises spontaneously in isolates of the *Aspergillus glaucus* group and is determined by a suppressive cytoplasmic factor (Jinks, *J. gen. Microbiol.*, *21*, 397, 1959). A recurrence of this condition in isolates of *A. amstelodami* made a reappraisal of the phenotype and its transmission possible. Vgd strains are characterised by irregularly growing colonies, a stop-start mode of growth in tubes and low conidial viability. Their cytochrome spectrum shows an excess of cytochrome *c* and a deficiency of *a*. These properties suggest that Vgd results from a mitochondrial mutation similar to *poky* and *stp* in *Neurospora crassa*.

The transfer of vegetative death to wild type (Vgd^+) strains through heterokaryosis is inhibited when donor and recipient are incompatible. Individual heterokaryon incompatibility loci differ in degree of inhibition, *het B* completely prohibiting transfer while *het A* reduced it to 21·4% of the compatible rate. A Vgd strain with yellow cleistothecia was mixed with a heterokaryon incompatible, Vgd^+ recipient which carried a marker producing orange cleistothecia. Hybrid cleistothecia of both yellow and orange type were selected and the transmission of Vgd determined. The incidence of Vgd colonies from orange cleistothecia was 1·1% as compared with 40·5% from yellow cleistothecia. These results are consistent with maternal inheritance of Vgd, and suggest that sexual plasmogamy in this species occurs between differentiated gametangia.

A NEW KIND OF DONOR STRAIN IN *STREPTOMYCES COELICOLOR*

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IF strains of *Streptomyces coelicolor* harbour a plasmid, SCP1 (A. Vivian, *J. Gen. Microbiol.*, *69*, 353, 1971). UF strains arise from IF by loss of SCP1 (D. A. Hopwood, R. J. Harold, A. Vivian and H. M. Ferguson, *Genetics*, *62*, 461, 1969). NF strains behave as donors when crossed with IF and UF strains (A. Vivian and D. A. Hopwood, *J. of Gen. Microbiol.*, *64*, 101, 1970).

An indirect selection procedure was used to obtain a new kind of donor strain from an IF strain (A. Vivian and D. A. Hopwood, *J. Gen. Microbiol.*, in press, 1973). The new donor strains differ from NF strains in their behaviour when crossed with UF strains. The chromosome fragments donated by NF strains to UF do not have constant ends, whereas those donated by the new donors have one constant end and one variable end.

The new donors are often unstable, segregating numerous IF and UF variants. It is possible that this new kind of donor strain arises by an interaction between SCP1 and the chromosome.

TWO MAJOR GENETIC AND PHYSIOLOGICAL CLASSES OF COLICIN FACTORS: THEIR PHYLOGENETIC SIGNIFICANCE

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Colicin (Col) factors, the autonomous bacterial plasmids specifying the antibiotics named " colicins ", are usually placed in a number of classes by the host range of their colicins. However, the effect of the chromosomal *recA* allele on colicin titres and the proportion of total colicin which is cell-bound distinguish two major classes of factors (K. G. Hardy and G. G. Meynell, *Proc. Biochem. Soc.*, 1972, *127*, 69P). One class contains ColE factors and ColK-235. The other class contains ColB, I and V factors.

The two classes are also distinguished by: the magnitude of colicin titres in *recA*$^+$ hosts; distinctness of their lacunae; molecular weight of the Col factor; presence or absence of a sex factor; maximum number of copies per chromosome; dependence on bacterial polymerases I and III (D. T. Kingsbury and D. R. Helinski, *Biochem. Biophys. Res. Commun.*, 1970, *41*, 1538; W. Goebel and H. Schrempf, *Ibid.*, 1972, *49*, 591); and by the chromosomal loci determining colicin tolerance or resistance (*tolA*, *tonB*).

Naturally-occurring Col factors may therefore fall into " EK-like " and into " BIV-like " classes. Conceivably, each class is separately descended from a single ancestor as has been suggested for F-like and I-like bacterial sex factors (E. Meynell, G. G. Meynell and N. Datta, *Bact. Rev.*, 1968, *32*, 55).

BY-PASS AND TRANSLATIONAL SUPPRESSORS OF A METHIONINE MUTANT IN THE FUNGUS *COPRINUS LAGOPUS*

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A mutation in the *me*-1 gene of Coprinus can be suppressed by five different recessive suppressor genes (Lewis, *Genet. Res., Camb.*, *2*, 141, 1961). Two of these *su*$^+$ genes, *su*-1 and *su*-2, complement when combined in a heterozygous dikaryon so that the *su*$^+$ phenotype is no longer expressed. The remaining three *su*$^+$ genes, *su*-3, *su*-4 and *su*-5, whilst complementing both *su*-1 and *su*-2, fail to complement each other in any combination. It has been suggested that non-complementation between recessive *su*$^+$ genes in fungi is a predictable property of missense translational *su*$^+$ genes specifying modified tRNAs with altered codon specificities (Todd and Casselton, *Heredity*, *28*, 274, 1972). Since *su*-1 and *su*-2 do complement it follows that the mechanism of suppression by these two genes is different from that of *su*-3, *su*-4 and *su*-5. Evidence will be presented which shows that the activity of *su*-3 and *su*-5 is allele specific, as expected of a translational suppressor mechanism, whereas that of *su*-1 and *su*-2 is allele unspecific. *su*-1 and *su*-2 are thought to act by causing the *me*-1 lesion to be by-passed so that the function of the *me*-1 protein is no longer required.

MUTATIONS, BASE-CHANGES AND GENE CONVERSION

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Base-changes in DNA do not necessarily cause abrupt, phenotypic changes recognisable as mutations. Two reasons for this are: degeneracy of the genetic code, so that a base-change may alter one codon for an amino acid to another codon for the same amino acid, and the ability of polypeptides to undergo certain amino acid changes without loss of function. Fully functional polypeptides, with or without identical amino acid sequences, may therefore be coded for by genes (alleles) which differ at one or more positions in their base sequence.

Two strains of an organism may be phenotypically wild-type for a given character and seem genotypically identical, showing no segregation in crosses. During crossing, however, the presence of an undetected base-change in one strain could lead to the formation of mis-paired bases at that site in hybrid-DNA. This mis-pairing could affect the conversion properties of nearby, known, segregating alleles when included in a cross. Such unsuspected mis-pairings may be relevant to such phenomena as polarity in conversion, and fine structure map expansion for which " marker effects " have been proposed.

PYRIMIDINE-DIMER EXCISION AND ULTRA-VIOLET LIGHT STIMULATED RECOMBINATION IN RECOMBINATION-DEFICIENT STRAINS OF *E. COLI* K12 REC B.

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The yields of Pl-mediated transductants in mutants of *E. coli* K12 with mutations in either the *rec*B or *rec*C loci, which appear to specify the structure of the ATP dependent nuclease, are usually only about 1% of those obtained in *rec*$^+$ strains. Irradiation of the transducing phage lysate with UV increased the yield of transductants about 3 fold in the *rec*$^+$ strain AB1157 but 30–50 fold in strain AB2463 *rec*B$^-$. The yield varied sharply with dose and was maximal near doses that reduce the phaque forming ability of P1 to 1%. X irradiation had a much slighter effect. No such increase was observed in the double mutant NH4275 *rec*B *uvr*A.

The linkage of unselected markers in these crosses was also determined and shown to be the same in the *recB* mutant as in its *rec*$^+$ parent. It is suggested that these data indicate that recombination in the *rec*B mutant is blocked at an early stage in the process but once initiated is normal, and that nicks introduced by the excision repair system can act to stimulate recombination for mutants that lack the *rec*B/C nuclease.

MUTABILITY OF *ESCHERICHIA COLI* AFTER INTRODUCTION OF AN IRRADIATED PLASMID

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One way of explaining some of the anomalies of UV mutagenesis in bacteria is to postulate a UV-induced, error-prone repair pathway. Witkin (1973, *Genetics*, in press) has developed such a hypothesis and used it to account for the wild-type level of induced mutations per survivor in *pol* strains and the dose-square kinetics of mutation induction at low to moderate doses of UV. Since the basis of her hypothesis is that the UV-inducible functions of UV mutagenesis, prophage induction, filamentation of *lon* strains, and UV reactivation of lambda bacteriophage are all expressions of the same inducible error-prone repair system, a method of testing the hypothesis became apparent. It is known that the last three functions mentioned, those of prophage induction, filamentation and UV reactivation can all be induced indirectly in an unirradiated recipient following introduction of an irradiated episome. We have performed experiments designed to show whether mutations, or hypermutability to low doses of UV, can be indirectly induced in the same way in unirradiated recipients, results which might be expected if all the afore-mentioned functions are indeed expressions of a common UV-induced system.

NONSENSE SUPPRESSORS WITH UNUSUAL PROPERTIES

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Nonsense suppressors exhibiting novel phenotypic characteristics were isolated and described by Bridges, Dennis and Munson (*Mol. Gen. Genet.*, *107*, 351–360, 1970) in investigating the interconversion of amber and ochre suppressors in *Escherichia coli* WU36–10. This strain has a tyrosine requirement which can be suppressed by ochre suppressors and a leucine requirement which can be suppressed by amber suppressors. We have found two genetically distinct ochre suppressors, classifying on phage testing

as *sup-2*, one of which is obtained by interconversion from amber *sup-2* whilst the other is not. The second ochre suppressor, lacking a corresponding amber suppressor, also maps in the *sup-2* region. A strain containing the interconverted ochre suppressor mutates at high frequency to a further additional amber suppressor which exhibits different phage suppression properties from amber *sup-2*.

AMINO ACID ANALOGUES AS MUTAGENS IN EUKARYOTES

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The amino acid analogues, *p*-fluorophenylalanine (PFP) and ethionine (ETH) are highly mutagenic in *Coprinus lagopus* and in the nuclear genes of *Saccharomyces cerevisiae*. Effective treatments with the analogues do not reduce viability.

By using different *Coprinus* mutant test systems, the analogues are mutagenic through single base changes. By using a variety of mutants which are resistant and sensitive to the analogues and which incorporate different proportions of the analogue into the protein and ethylate the bases of nucleic acid in different proportions, it is concluded that PFP is mutagenic primarily through its incorporation into protein and ETH primarily through its ethylation of nucleic acids.

PFP treatment followed by ultraviolet irradiation, has a highly synergistic effect. With ultraviolet sensitive strains the mutagenic effect of PFP is greatly reduced. This implies that PFP is mutagenic by virtue of its incorporation into DNA replication and repair enzymes.

The analogue PFP is not mutagenic in 2 strains of *E. coli*, in *Pseudomonas aeruginosa* and in the mitochondria of *S. cerevisiae*

NEW CLASS OF DOMINANCE MODIFIER GENES IN *CORPRINUS LAGOPUS*

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Wild type *Coprinus lagopus* is resistant to canavanine; this resistance is dominant to sensitivity in dikaryons and diploids. Wild type is sensitive to parafluorophenylalanine (PFP) and resistant mutants are recessive. A rigorous selection for dominant resistance in auxotrophically balanced dikaryons resulted in two new recessive resistance genes *pfp10* and *pfp11* and a new class of two specific dominance modifiers. Without the modifier the resistance genes are recessive in dikaryons; with one dose of the modifier the resistance genes are fully dominant in the dikaryon. In the diploid the resistance gene is recessive even with two doses of the modifier gene. The only other detectable effect of the modifier is to reduce the growth rate in haploids.

ALGEBRA FOR A FIELD-MODEL OF INTENSE ENDOCYCLIC SELECTION IN ITALIAN STRAINS OF *MANIOLA JURTINA*

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Scali and Masetti (in press) have reported that embryos in some Italian strains of *Maniola jurtina* L. (Lepidoptera: Satyridae) have cytologically demonstrable deviations from 1:1 sex ratio. These are usually significantly in favour of females. Sampling of larvae in the field and estimates of pre-aestivation imagines in June show that the early female advantage is reversed and males heavily preponderate at copulation time. Scali has shown that during aestivation selection of 60–80% occurs against more spotted female imagines. A lesser selection on spot-placing patterns also occurs

in aestivating Italian females. McWhirter (in prep.) finds that an aestivating Italian colony (not studied cytologically) has strong heritability of female spot-number.

These intensive endocyclic selections make necessary a development of classical algebra. Estimates of original embryonic numbers place a limit on the intensity of selection which can be supported in stable populations. One or more genetic components in the elimination of nearly all females can be calculated. Truly " accidental " death must be rare where selective elimination of females reaches the 90–95% zone before the later imaginal eliminations occur during aestivation.

A simple algebra accommodating parameters of population-structure as well as estimates of selective elimination is presented.

ASSIGNMENT OF HUMAN RED CELL ACID PHOSPHATASE GENE LOCUS TO THE SHORT ARM OF CHROMOSOME 2

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A reciprocal translocation between the short arm of chromosome 2 and the long arm of chromosome 5 has been found in the mother and sister of a severely retarded child with developmental malformations. The child has an unbalanced translocation with duplication of part of chromosome 5 and deletion of part of chromosome 2. Evidence will be presented that the child has only the paternal allele at the red cell acid phosphatase (AcP) locus. It is postulated that the ACP gene locus lies within the small deleted segment of the short arm of chromosome 2 defined by Giemsa banding techniques.

RECOMBINATION IN BACTERIOPHAGE LAMBDA

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Bacteriophage λ has two different pathways whereby genetic recombination can occur.

1. The *red* pathway permits recombination between any two different genetic markers (generalised recombination). Two genes of the *red* pathway have been identified *redα* and *redβ*. The products of both genes have been purified; *redα* specifies an exonuclease active on double stranded DNA and *redβ* codes for a protein which facilitates binding of the exonuclease to DNA. A third gene (*gamma*) has been identified. The product of this gene has little effect on *red* mediated recombination but acts to reduce recombination via the host *rec* system by inactivating the *rec*BC nuclease (exonuclease V).

2. λ is also capable of site specific recombination across the attachment site. This is the system whereby λ integrates into, and excises from, the host chromosome, but it is also able to direct recombination between markers on opposite sides of the attachment site in vegetative phage crosses. Two genes have been identified. The *int* gene is necessary for both integration and excision while the *xis* gene product is necessary only for excision. Neither gene product has been identified *in vitro*.

DNA REPLICATION IN *POLA RECB* AND *POLA RECA* MUTANTS OF *ESCHERICHIA COLI*

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An inter-relationship between the processes of DNA replication, repair and recombination is indicated by the non-viability of *polA recA* and *polA recB* double mutants. Strains carrying the temperature sensitive mutation *polA12* together with *recB21* are normally non-viable at 41° C. but can be " rescued " by *sbcA* and *sbcB* suppressor mutations. Strains carrying *polA recB sbcA* are characterised by the appearance of a new ATP-independent DNase and strains carrying *polA recB sbcB* by the absence of exonuclease I.

It is possible that the non-viability of *polA12 recB* and *polA12 recA* mutants at 41° C. might be associated with their inability to remove, or control the removal of priming RNA.

GENETICAL AND BIOCHEMICAL STUDIES ON GENETIC RECOMBINATION, REPAIR AND REPLICATION IN *USTILAGO MAYDIS*

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A review will be presented of recent progress by several investigators of the genetic and biochemical control of recombination, repair and replication in a simple eukaryotic organism, the yeast-like fungus *Ustilago maydis*. In general, the approach involves isolating and characterising mutants defective in these processes and studying the DNA enzymology of wild type strains, in order to identify the biochemical defect of the mutants.

Some radiation sensitive mutants have abnormal genetic recombination and meiosis. These appear to be capable of normal excision of UV-induced pyrimidine dimers from DNA. Others which are defective in this excision process have normal recombination. Two DNases have been purified and characterised, and DNase deficient mutants have been shown to be defective in genetic recombination. A temperature sensitive mutant blocked in DNA synthesis at the restrictive temperature contains a heat labile DNA polymerase. This enzyme does not appear to be required for repair and recombination. It and at least two other DNA polymerases are being purified and characterised. The hope is that when enough mutants with particular genetic properties have been shown to lack well characterised enzymes, information will be gained about the underlying molecular mechanisms which govern the behaviour of genetic material.

GENETIC EXCHANGES CAUSED BY CERTAIN TYPES OF STRUCTURAL DAMAGE IN DNA

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The frequency of genetic recombination in mitotic and meiotic systems can generally be increased by exposing the organisms to radiation or to chemical mutagens. Evidence from bacterial and phage systems indicates that increased recombination is caused by structural damage in DNA molecules.

Two systems have been used in studies on induced recombination. In the first, sister exchanges induced by ultraviolet irradiation in *E. coli* were detected by means of density labels in the DNA. In the second system, *E. coli* K12 lysogenic for phage λP80 was infected with normal or experimentally treated phage λP3, and the yields

of P+ recombinant prophages were determined many generations later following induction and the release of mature phages. This system had the merit that the replication of the infecting phages was repressed by λ immunity, but replication of the prophages occurred as the bacterial chromosomes underwent replication.

Only certain types of structural damage in the DNA were found to cause exchanges efficiently. Pyrimidine dimers in otherwise intact duplex DNA do not cause exchanges if the DNA in which they are contained is not replicated. Nor is recombination caused by the transient single strand gaps formed during excision repair. However, if the DNA containing pyrimidine dimers is allowed to replicate, the newly synthesized daughter strands terminate at each dimer leaving a gap before a new strand is initiated. Dimers opposite post-replication gaps appear to initiate genetic exchanges with high efficiency.

The treatment of phage λP3 with 360 nm light in the presence of the sensitising agent trimethyl-psoralen produces interstrand cross-links and monoadducts, and causes genetic exchanges efficiently in the absence of replication provided the cells are excision proficient and not *uvrA*. By analogy with a pyrimidine dimer and post-replication gap, it is thought that an exchange can be caused by an interstrand cross-link after one side of the cross-link has been excised from one strand. This leaves a single strand gap with a cross-link still attached by one arm to the opposite strand.

Certain products formed by ultraviolet light of wavelength 254 nm, possibly pyrimidine adducts or photohydrates, cause exchanges efficiently in the absence of replication even in excision deficient cells (*uvrA*$^-$).

These experiments lead to the conclusion that phage or bacterial DNA molecules containing certain types of structural damage can interact with undamaged homologous molecules in such a way as to initiate genetic exchanges.

GENETIC RECOMBINATION IN BACTERIOPHAGE ØX174

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Genetic recombination in bacteriophage φX174 usually occurs between two parental replicative form (RF) DNA molecules early in the infection process. The host *recA* gene product is required; none of the nine known φX174 cistron products is essential. The products of a single recombination event are non-reciprocal and asymmetric: only one of the parental genotypes and one recombinant genotype are recovered.

In host cells carrying the *recA*+ gene the formation of φX174 genetic recombinants is stimulated up to 50-fold by subjecting the virus particles to UV before infection or by starving the host cell for thymine during infection; in a *recA* host strain no such increase is observed.

RF DNA molecules formed *in vivo* from UV irradiated bacteriophage φX174 consist of an intact viral strand and a partially complete complementary strand extending from the point of origin to a UV lesion. RF DNA molecules formed in thymine-deficient hosts during thymine starvation have complete viral and complementary strands which contain random single-strand breaks or gaps. These observations suggest that single strand " breaks " are intermediate structures in the formation of φX174 genetic recombinants mediated by the host *recA*+ enzyme.

Examination by electron microscopy of the DNA structures found in *Rec*+ cells suggests that branch migration occurs during φX174 recombinant formation.

An alternative, less efficient, mechanism which requires an active φX174 cistron A product is observable in the absence of the host *recA* gene product.

INTEGRATION OF F-FACTORS UNDER THE INFLUENCE OF Mu-1

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Bacteriophage Mu stimulates greatly chromosome-mobilisation by F-factors in a *recA* strain and causes also very stable integration at any site of the bacterial chromosome of an Fts*lac* episome in a *recA* strain. The F-factor is not integrated at its own normal integration point(s) but randomly so that many of the integrations lead to sterile donors. When the strains are made *rec*$^+$ the integrated episomes are segregated with a frequency of nearly 5% in one type (I) and with a frequency of nearly 0·025% in another type (II). Moreover type II integrations have a spontaneous phage production which is 10^4 times lower than type I integrations.

An Fts*lac* (Mu) episome, resulting from segregation of a Rec$^+$ derivative of a type I integration, was used to determine the orientation of Mu prophages in the *trp*-operon by chromosome mobilization experiments (a Mu-prophage can have 2 possible orientations, indicated with plus and minus respectively). It was found that a minus Mu prophage could be mobilised in two different directions, suggesting that part of a plus Mu prophage has the same orientation as the minus Mu prophage. When the Fts*lac* (Mu) is integrated in the minus Mu prophage, Hfr's were isolated which transfer in the same direction as the Hfr's which are due to integration of Fts*lac* (Mu) in a plus Mu prophage. Probably stable integrations take place preferentially at that part of Mu which has the same orientation in both types of prophages.

The Hfr's which are due to integration of the Fts*lac* (Mu) in the minus Mu-prophage produce very little phage when made *recA* and behave similarly but not identically to the type II integrations which are formed by the Mu-promoted integration of F-factors in *recA* strains. The experiments suggest that two Mu-molecules can interact with each other at a structural site which can invert and which is probably identical with the G-loop area which has been shown to be present in Mu-DNA by electron microscope studies.

After the interaction, attachment sites, one for the F-factor and one for the chromosome, can be formed in several ways and theoretically four different types of integrations, including type II integrations, should be found.

It can be shown that none of the Mu genes known until now are located on the supposed structural site.