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ABSTRACTS of papers presented at the HUNDRED AND NINETY-EIGHTH MEETING of the Society held from 28th to 30th March, 1983 at the UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY.

UNIDENTIFIED READING FRAMES IN THE MITOCHONDRIAL GENOME OF *ASPERGILLUS NIDULANS*

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Eight reading frames have so far been characterised in the mitochondrial genome of *Aspergillus nidulans* by us or by the group of H. Küntzel. Two of them are homologous to the URFs 1 and 4 of the mammalian mitochondrial genome. A third, URF coding for a polypeptide only 48 residues in length, is between a cluster of two tRNA genes and the gene coding for ATPase subunit 6. The tRNA gene proximal to the URF, the spacer and the amino terminal portion of this URF is repeated elsewhere in the mitochondrial genome.

TRANSFORMATION IN *ASPERGILLUS NIDULANS*

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We shall describe a system to study transformation *A. nidulans*. This comprises an *A. nidulans* cloned gene and vectors carrying *A. nidulans* origins of replication or the *A. nidulans* ribsomal repeat. The recipient strain carries a well characterised deletion in the relevant gene. The different parameters involved in obtaining a successful transformation system will be discussed.

SEQUENCE CONSERVATION AND SECONDARY STRUCTURE IDENTITY BETWEEN SOME NUCLEAR AND MITOCHONDRIAL INTRONS

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A group of sequences is strictly conserved between some fungal mitochondrial introns and the precursor of the large (nuclear coded) cytoplasmic RNA of two *Tetrahymena* species. Using these conserved sequences as nucleation points, almost identical secondary structures can be drawn for all these introns. These structures align the splice points to one phosphodiester

bond "making ends meet" according to the model of Davies *et al.*, *Nature*, (in press). One of the *Physarum polycephalum* nuclearly coded rRNA introns and the *Chlamydomonas reinhardii* chloroplast rRNA intron also possess some of the features first described for some mitochondrial introns. There may be some common features, involving an internal guide RNA (Davies *et al.*, *Nature* in press) in the mechanism of splicing of RNA precursors coded by some mitochondrial, chloroplast, and extra chromosomal nuclear genes.

MOLECULAR AND GENETIC STUDIES ON THE TOL (TOLUENE AND XYLENE DEGRADING) PLASMID OF *PSEUDOMONAS PUTIDA*

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We shall present evidence that the loss of a specific 40 kb segment of the TOL plasmid bounded by a 1·4 kb direct repeat is a *rec*-independent event in both *Pseudomonas putida* and *Escherichia coli.*

We shall also present data on cloned fragments of the TOL plasmid and *R*-plasmid–TOL recombinants. Functions have been mapped by deletion analysis, transposon mutagenesis and Southern hybridization. We have initiated a sequence analysis of the toluate oxygenase (*xylD*) gene.

THE STRUCTURE AND AMPLIFICATION OF THE GLUTAMINE SYNTHETASE GENE IN VARIANT CHO CELLS

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We have amplified the gene for glutamine synthetase (GS) by multistep selection of CHO KG1 cells grown in the presence of methionine sulphoximine. The mutant cell line KG1MSC4-M has amplified approximately 50 kbp of DNA and overproduces a polypeptide of molecular weight 43,000 which co-migrates with purified GS.

A DNA band of 8·2 kbp from a C4-M digest has been cloned into pUC-9 to produce recombinant plasmids pGS-1 and pGS-2. pGS-1 hybrid selects mRNA which translates to produce a 43,000 molecular weight protein. This protein co-migrates with purified GS. We are currently determining GS gene copy number, GS mRNA abundance and the structure of the GS gene within the amplified DNA unit.

NATURALLY-OCCURRING CONJUGATION IN THE OBLIGATE METHANOL-UTILISING BACTERIUM *METHYLOMONAS (METHANOMONAS) METHYLOVORA*

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We have shown that *Methylomonas methylovora* contains a plasmid with a molecular weight of 88×10^6 Daltons (Monteiro *et al.*, *FEMS Microbiol. Letters, 15*, 235–237, 1982). Mutant strains have been isolated which are resistant to a variety of inhibitors including ampicillin, chloramphenicol, fluorouracil, lincomycin, mercuric ions, naladixic acid, rifampicin, streptomycin, trimethoprim, vibramycin and valine.

Multiply-marked strains have been prepared and pairs of strains mated on agar surfaces. Transfer of the fluorouracil resistance marker occurred at a frequancy of $2{\cdot}5 \times 10^{-4}$ whereas the spontaneous reversion frequency was $4{\cdot}3 \times 10^{-7}$. Analysis of the fluorouracil resistant exconjugants showed that 75 per cent of these strains had also received the ampicillin and streptomycin markers. This demonstrates that the plasmid is conjugative, as would be expected from its size. Current research is directed towards optimising the conjugation system and analysing the plasmid further.

THE PRODUCTION OF PARA-AMINOBENZOIC ACID AS A POSSIBLE MECHANISM OF SULPHANILAMIDE RESISTANCE IN *METHYLOMONAS METHYLOVORA*

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The obligate methylotroph *Methylomonas methylovora* (NCIB 11376) which contains a plasmid with a relative molecular mass of 88×10^6 Daltons (Monteiro *et al.*, *FEMS Microbiol. Letters, 15,* 235–237, 1982) has also been shown to be naturally resistant to 4 mM sulphanilamide on solid media. During viable count estimations by the Miles–Misra technique, it was noticed that high cell densities could crossfeed low densities of cells resulting in an apparent increase of resistance. Culture supernatants from *Methylomonas methylovora* grown in liquid minimal media were able to partially overcome the para-aminobenzoic acid (PAB) requirement of a *paba* mutant of *Aspergillus nidulans*. This suggests that sulphanilamide resistance is associated with the overproduction of PAB. This mechanism has been reported in *Staphylococcus aureus* (White and Woods, *J.G.M., 40,* 255–271, 1965).

Current research is investigating the relationships between growth phase and PAB production in the presence and absence of sulphanilamide. It is also hoped to identify the genetic basis of sulphanilamide resistance which has been shown in other bacteria to be plasmid determined (Wise *et al.*, *P.N.A.S., 72,* 2621–2325, 1975).

THE SWITCH OF THE SPECIFICITY OF THE R124 RESTRICTION AND MODIFICATION SYSTEM

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The incFIV plasmid R124 codes for a unique restriction and modification (R–M) system and the derivative plasmid R124/3 codes for an R–M system with a different specificity. There is evidence that both R–M systems are coded for by both R124 or R124/3 and that normally only one set is expressed. The discovery that the R124 or R124/3 genes can be transferred to R-plasmids enabled both sets of genes to be introduced into a cell. These experiments showed that there is a genetic switch of specificity. Analysis of the DNA from these strains shows that this switch involves DNA rearrangements and that the switch is not a simple inversion of DNA.

A NOVEL CLASS OF RESTRICTION-DEFICIENT MUTANTS INVOLVING DNA REARRANGEMENTS

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R124 is an incFIV plasmid which confers tetracycline resistance and carries a unique restriction and modification (R–M) system. R124/3 is a derivative plasmid that codes for an

R–M system of a different specificity. It has been shown that F^+ produces restriction-deficient mutants of these plasmids independently of the *rec*A gene product. These mutants carry extensive deletions and other DNA rearrangements which will be described in detail. These mutants are unstable and can undergo further DNA rearrangements to produce stable derivatives. All of these rearrangements are independent of *rec*A and of the continued presence of F^+. One such rearrangement is the transfer of the R–M genes to F^+ and this has enabled us to show the presence of a genetic switch of specificity on R124 and R124/3 which is described in a separate communication.

SEQUENCING OF CDNAS FROM PEA

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The major storage proteins of pea (*Pisum sativum*): legumin, vicilin and convicilin are an important model system for the control of gene expression in plants, being synthesised in a single tissue at a clearly defined stage of development. The nutritional value of these proteins, especially vicilin, is limited by a low level of the sulphur-containing amino acids. Modern molecular biological techniques offer a suitable potential means of improving the protein quality.

As a first step in understanding this gene system, we have cloned cDNAs coding for several of the proteins and have presented the DNA sequence of two coding for legumin (Croy *et al.*, *Nature 295*, 76–79, 1982). We now present the sequence of a longer legumin cDNA and of cDNAs coding for several variants of vicilin. Some unusual features are noted, including a codon usage pattern somewhat different from that observed for animals. These features are shared by some other plant genes. We also present evidence for the existence in the polypeptides of a leader sequence and several post-translational proteolysis sites. One of the legumin cDNAs contains a deletion of a sequence coding for an extremely polar region of the polypeptide. It is not yet clear whether this represents a true heterogeneity, or whether it is an artefact.

GENETIC DIVERSITY IN BACTERIA

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A comprehensive theory of diversity must encompass variation in bacteria, but discussion of diversity often omits bacteria. There are several reasons. Firstly, evolutionists are often unaware of the endless variation shown by bacteria. Secondly, bacteria are haploid, which puts them rather outside the line of thought which culminated in the concept of genetical polymorphism. Thirdly, it is difficult to know what significance to attach to bacterial species; in particular, we do not know the extent of gene flow within and between bacterial species.

The vast range of variation shown by *Escherichia coli* and its relatives will be illustrated.

About 50 per cent of *E. coli* isolates use sucrose as a carbon source. The strain K12 does not. Only in about 3 per cent of strains are sucrose genes carried on conjugative plasmids. Sucrose genes transferred from wild strains to K12 take a constant location near min 51 on the K12 linkage map (Alaeddinoglu and Charles, *J. Gen. Microbiol.*, *110*, 47, 1980).

About 20 per cent of strains use sorbose. The sorbose genes from wild strains take a location near min 91 on the K12 linkage map (Woodward and Charles, *ibid.*, *128*, 169, 1982).

About 18 per cent of wild strains use ribitol and arbitol, and about 35 per cent use galactitol, and few if any use all three. When the ribitol and arabitol genes are transferred to K12, they apparently displace the galactitol genes at min 46, suggesting competition for the same chromosomal region (Woodward and Charles, *ibid.*, in press).

Woodward and Charles proposed the concept of an ideal *E. coli* map with different wild strains displaying different samples of genes from the ideal map. The implications of constant locations will be discussed.

If a strain is sucrose negative, does it lack all traces of sucrose genes, or possess defective genes? Analysis showed that sucrose-positive strains possess a gene for a sucrose permease and a gene for invertase, and associated control regions. Some sucrose negative wild strains

mutate to use sucrose. They are "defective" only in the permease gene, and the "defect" seems easily reversible. On the other hand some strains possess an an active permease, and are defective in the invertase gene, or lack it altogether, because they do not mutate to form invertase (Hill and Charles, *Soc. Gen. Microbiol. Quarterly*, 7, 82, 1980).

Possible models for *E. coli* variation will be discussed.

ON THE EVOLUTIONARY COMPLEXITIES OF PLANT–ANIMAL INTERACTIONS

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We are attempting to explain why *Lotus corniculatus* is polymorphic for cyanogenesis. Recently we have been dissecting habitats in which the relative proportions of cyanogenic and acyanogenic individuals vary over short distances, at least in some habitats.

One complication is that plants often show genotype x environment interaction for *leaf* cyanogenesis with the result that the pattern of distribution of the cyanogenic morphs within a group of plants can change during the course of a year without any other change in the composition of the group. Furthermore, there is a separate and partly independent polymorphism for cyanogenesis in *petals*; all 16 possible phenotypes have been detected in some habitats.

The leaves and the flowers sustain two distinct guilds of insect herbivores and although these animals can cause considerable damage to plants they are normally not selective. Molluscs, on the other hand, do show selective feeding both on leaves and on petals. The expected second order interaction between the plants, the animals and very local differences in the environment reveals that there will be no simple explanation of the cost to a plant of being cyanogenic.

HOST-RANGE MUTANTS OF THE PROMISCUOUS PLASMID R300B

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We have isolated Tn1 insertion mutants of R300B and tested them for their ability to be mobilised by RP4 into eight different bacterial species. Several classes of mutants were isolated. These mapped in separate regions of the R300B genome.

THE STABILITY OF COLE1-RELATED PLASMIDS IN *ESCHERICHIA COLI*

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Despite our considerable knowledge of the replication mechanism of ColE1-related plasmids and their extensive use as the plasmid cloning vectors for *in vitro* genetic manipulation, little is known about the factors that determine plasmid stability in bacterial populations.

Our studies of copy number and plasmid stability using ColE1, derivatives of it, and the plasmid cloning vectors pBR322 and pAT153, have shown that many factors determine plasmid stability. These include:

1. *The ability of plasmids to exist as multimers in cells.* Multimers are less stable than monomers, probably because of their lower copy number. Bacterial mutations that reduce plasmid recombination stabilise plasmids when they are initially present as monomers. Similarly site-specific recombination systems that efficiently convert multimers to monomers, stabilise plasmids.

2. *The mean and variance of plasmid copy numbers.* The observed instability of some ColE1-related plasmids is consistent with the idea that they are stochastically partitioned at cell division. In general, a higher mean copy number results in higher stability. However, indirect evidence suggests that the variance about the mean is itself variable and is important

in determining stability. For example, some very high copy number plasmids (*i.e.* high *population* mean) may be unstable as a consequence of having a very high variance of copy number in the population: a low copy number *tail* may continuously segregate plasmid-free cells.

3. *Competition between plasmid-containing and plasmid-free cells.* The relative disadvantage/advantage of plasmid-containing cells compared to plasmid-free cells varies enormously from plasmid to plasmid. Competition is a major contributor to observed segregation rates.

We have been unable to find any evidence for a sequence within ColE1 analogous to *par* of pSC101 (Meacock and Cohen, *Cell, 20,* 529–543, 1980).

STUDIES ON THE EXPRESSION OF *NIF:LAC* GENE FUSIONS IN *AGROBACTERIUM TUMEFACIENS*

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Since *Agrobacterium* is a rhizosphere bacterium, and is capable of infecting a wide variety of plants, unlike the *Rhizobium* species, it would be useful if the former could be converted into a nitrogen-fixing bacterium. Preliminary investigations carried out at the Brighton ARC Unit showed that the well-investigated *nif* gene cluster of *Klebsiella pneumoniae* produces only inactive nitrogenase when introduced into *A. tumefaciens* (Dixon *et al., Nature 260,* 268, 1976). In order to investigate which *nif* gene(s) experience transcriptional difficulty in *A. tumefacien,* we are introducing into this bacterium a series of plasmids carrying different *nif* promoters fused with the *E. coli lac* structural gene; the transcription of the *nif* promoters is being assayed by measuring the production of β-galactosidase under aerobic and anaerobic conditions, with and without fixed nitrogen. The data obtained so far indicate that the promoters of *nif H D K* (structural genes for nitrogenase) and *nif L A* (general regulator of the *nif* system) expresses well in *A. tumefaciens* and are less sensitive to aerobic conditions; also, they are almost insensitive to the presence of fixed nitrogen, unlike their original host.

CLONING OF BACTERIAL D-XYLOSE ISOMERASE IN YEAST

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The putative structural gene for D-xylose isomerase has been subcloned from pCL10-15 (a Clarke and Carbon ColE1–*Escherichia coli* DNA hybrid plasmid) into the yeast–*E. coli* shuttle vector YEp13. The resulting hybrid plasmid, pAM101, has been shown to complement the *xyl-5* mutation in *E. coli* JA200, which is defective in D-xylose isomerase.

The yeast *Saccharomyces cerevisiae* is unable to utilise D-xylose but it readily assimilates and ferments the keto-isomer D-xylulose. Transformation of this yeast has been achieved with pAM101. The transformants are being characterised with regard to expression of the D-xylose isomerase gene under oxidative and fermentative conditions.

THE *UVRD* GENE *E. COLI* ENCODES A HELICASE

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We have recently identified the *uvrD* gene product as a 73 kd protein. Subcloning the *uvr*D gene into amplification vectors results in elevated levels of the product amounting to 3 per cent of total protein. This amplification permits a simplified procedure which yields an enzyme with DNA–dependent ATPase and ATP-dependent helicase activities identical to those of helicase II.

SEQUENCING THE *E. COLI UVRD* GENE

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The *uvrD* gene has been subcloned from pHMA9 (*uvrD*$^+$) into phage M13 vectors and sequencing by the dideoxy chain termination technique. The amino acid sequence and predicted secondary structure will be presented.

ANALYSIS OF THE CHLORAMPHENICOL RESISTANCE DETERMINANT OF PLASMID R26

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The Cm resistance determinant of R26 is inducible and does not involve drug inactivation. Deletion and Tn5 insertion mutations show that Cm^r is encoded in a 2000 bp sequence of DNA. Experiments are in progress to identify the number of genes and their protein products as well as to understand the mechanism of regulation.

GENETIC ANALYSIS OF THE MERCURIC RESISTANCE DETERMINANT OF R100

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The *mer* genes of plasmid R100 were cloned in pBR322. The *merR*, *merT* and *merA* genes were mapped using Tn5 insertion and deletion mutations. Evidence was obtained for a new *mer* gene mapping between *merT* and *merA*. We are currently attempting to assign Hg-inducible polypeptides formed in minicells to *mer* genes.

MOLECULAR CLONING OF THE *PSEUDOMONAS* GENE CODING FOR CARBOXYPEPTIDASE G_2: NUCLEOTIDE SEQUENCE AND LEVEL OF EXPRESSION

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The gene coding for the therapeutic enzyme carboxypeptidase G_2 has been cloned in *E. coli* using pBR322. Sub-clones have been obtained which produce up to 5 per cent soluble protein (cf <0·1 per cent from the *Pseudomonas*), the enzyme being located in the periplasm. The entire nucleotide sequence of the structural gene has been determined, and the predicted protein sequence confirmed.

INTEGRATION OF R68·45 INTO THE GENOME OF A CYANOBACTERIUM RESULTS IN GENOME MOBILISATION

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R68·45(Ap^r, Km^r, Tc^r) was transferred from *Escherichia coli* to *Synechococcus* PCC301 by filter mating, and transconjugants resistant to ampicillin and kanamycin were selected. Although these transconjugants were cross-resistant to tetracycline the autonomous plasmid could not be detected. *Pst*I digestion of total DNA from two transconjugants revealed fragments which corresponded to R68·45 fragments and which were absent from the wild type *Synechococcus*. This suggested that R68·45 had integrated into a cyanobacterial replicon.

Co-integration with one of the indigenous plasmids was excluded since there was no change in the size of these in transconjugants. R68·45, then, must have integrated into the chromosomal DNA. Integration was confirmed by *Bam*HI-digestion of total DNA from transconjugants. This produced two unique fragments whereas the autonomous plasmid is cleaved to a single fragment. Southern hybridisation indicated that the integration site on the plasmid was close to, or coincident with, an IS*8* element.

Conjugation experiments using these transconjugants as donors in crosses with auxotrophic recipients yielded equivocal results, but this might have been due to the limited range of genetic markers available for analysis. Intergeneric crosses between these transconjugants and auxotrophic *E. coli* recipients resulted in the transfer of a *Synechococcus* gene which could complement a *his* mutant of *E. coli* This indicated that R68·45 can mobilise the *Synechococcus* genome. This system should serve as a useful method of chromosomal mapping in *Synechococcus* PCC6301 once sufficient genetic markers are available.

AN ANALYSIS OF *BACILLUS SUBTILIS* ENDO-β-1,3–1,4-GLUCANASE GENE EXPRESSION IN *ESCHERICHIA COLI*

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A rapid screening method has been developed for the detection of microorganisms with endo-β-1,4-glucanase activity. The assay is based on the ability of certain dyes to form insoluble coloured complexes with high molecular weight β-glucans. The use of this novel procedure is described to the screen for the isolation of an endo-β-1, 3-1,4-glucanase gene from a gene bank of *B. subtilis* DNA, prepared using an *Escherichia coli* bacteriophage λ host-vector cloning system. A 4 Kb Eco RI fragment containing the active gene was subcloned in both orientations in plasmid pBR325 and a restriction map of the DNA insert was determined for 22 restriction enzymes. A series of sub-cloning experiments established the position of the gene on the 4 Kb Eco RI fragment and a new recombinant plasmid was constructed which contains the endo-β-1, 3-1,4-glucanase gene on a 1·6 Kb Eco RI–Pvu I fragment. The gene is functionally expressed in *E. coli* as demonstrated by direct enzyme assays and SDS-polyacrylamide gel electrophoresis of ^{35}S-L-methionine labelled proteins made in maxicells. The localisation of the endo-β-1,3-1,4-glucanase protein in *E. coli* is now being investigated.

The enzyme endo-β-1, 3-1,4-glucanase, which is produced extracellularly by *B. subtilis*, specifically hydrolyses β-glucans containing β-1,3 and β-1,4 linked glucose units, such as lichenan and barley β-glucan. The importance of this enzyme to the brewing industry will be discussed.

HIGH EFFICIENCY EXPRESSION OF HETEROLOGOUS GENES IN THE YEAST *SACCHAROMYCES CEREVISIAE*

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We have constructed a series of plasmid vectors that allow the efficient expression of foreign genes in the yeast *Saccharomyces cerevisiae*. These vectors utilise the efficient promotor of the yeast *PGK1* (3 phosphoglycerate kinase) gene, together with a 2u plasmid replication origin and the yeast *LEU2* gene as a selectable marker. Both transcriptional and transcriptional/translational fusion vectors have been constructed.

A number of different heterologous genes have been inserted into these vectors and their expression in yeast examined. The human gene coding for interferon-alpha-2 is efficiently expressed in these vectors and the interferon synthesised represents between 2 and 8 per cent of total cell protein under optimal conditions. Both interferon and PGK-interferon

protein synthesised in yeast are inactivated by specific anti-interferon-alpha antibodies. Expression of the chromosomal glycolytic genes. The other heterologous genes successfully expressed in yeast using these vectors include both prokaryotic (*E. coli*–galactosidase gene) and eukaryotic (*Herpes simplex* thymidine kinase gene) genes.

Factors which may influence the efficiency of expression of foreign genes in yeast will be described. These include transcriptional (initiation and termination sequences), translational (codon usage, AUG nucleotide environment) and post-translational (protein stability) effects.

CONTRANSDUCTION OF AUXOTROPHIC MARKERS BY PHAGE SVI IN *STREPTOMYCES VENEZUELAE*

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Generalised transduction of auxotrophic markers in *Streptomyces* was first reproducibly demonstrated in *S. venezuelae* strain 13s using temperate phage SV1 (Stuttard, *J. Gen. Microbiol., 110,* 479, 1979; Stuttard, *J. Gen. Microbiol., 128* 115, 1982). The feasibility of localized hydroxylamine mutagenesis by transduction was also demonstrated in this system (Stuttard, *Zbl. Bakt. Abt. 1: Suppl., 11,* 533, 1981). Subsequently, the type of strain of *S. venezuelae,* ATCC 10712, was found to be better for mutagenesis, and a wide range of auxotrophic markers was tested for cotransducibility in this strain. The first example of cotransduction in *S. venezuelae* involved 3 independently-isolated histidine (*his*) markers (Stuttard, unpublished data).

Very recently, a group of cotransducible genes involved in aspartate amino acid biosynthesis was identified after hydroxylamine mutagenesis of transducing phages and transduction of a *lysA* mutant to prototrophy. Three different threonine (*thr*) markers were cotransducible with several different *lysA* mutations at frequencies of 36 to 62 per cent. One of the *thr* mutations was isolated after cotransductional (localised) mutagenesis while the other two came from existing stocks of mutants isolated after 8-methoxypsoralen-near UV mutagenesis.

The location of *thr* genes adjacent to *lysA* in *S. venezuelae* is different from the situation in *S. coelicolor* A3(2) where *lysA* is diametrically opposite to *thr* genes on the linkage map (Hopwood, *Bacteriol. Rev. 31,* 373, 1967), but is similar to analogous loci in *S. rimosus* and *S. bikiniensis* var *zorbonensis* (Friend and Hopwood, *J. Gen. Microbiol., 68,* 187, 1971).

AGE RELATED ANEUPLOIDY AND IRREGULAR CYCLICITY IN FEMALE MICE

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A rise in the frequency of aneuploidy occurs with maternal age both in man and the CBA mouse (Fabricant and Schneider, *Developmental Biology, 66,* 377–343, 1978). Numerous theories have been advanced to explain this maternal age effect, although none adequately do so. In this study an attempt has been made to distinguish between the effect of chronological ageing and the reproductive physiological age of the female.

Unilateral ovariectomy (OVX) in CBA mice was used to bring forward the period of irregular cyclicity and the cessation of fertility. These changes occurred approximately 3 months earlier in OVX compared with sham operated controls. Chromosome preparations of $3\frac{1}{2}$ day old blastocysts from OVX and sham operated females of various ages showed that an increase in aneuploidy with age occurred in both groups . The rise in aneuploidy for OVX however, occurred earlier than for sham controls. In both cases this rise preceded the onset of acyclicity.

HERPES SIMPLEX VIRUS PROMOTER SEQUENCES

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Herpes simplex virus (HSV) is a large DNA-containing virus whose genome is transcribed by cellular RNA polymerase II throughout infection. After infection of tissue culture cells, viral "immediate early" (IE) genes are the first to be expressed, and their products, the IE polypeptides, have important roles in controlling virus transcription at later times. We have investigated the DNA sequences important for regulation of transcription of one IE mRNA, IE mRNA 3, by construction of plasmids with specific deletions in the promoter region. In many cases, these altered promoters have been linked to the thymidine kinase (TK) structural gene and expression measured by enzyme assays.

Two distinct promoter elements have been identified:

1. A short upstream sequence is equivalent in efficiency to the normal TK promoter, but differs in the nature and position of the vital sequences. It is also unlike other eukaryotic promoters in these respects.

2. A DNA region further upstream strongly enhances expression from the shorter promoter. This region also contains sequences which respond to positive regulation by a component of the virus particle.

THE SEQUENCE AND HIGH LEVEL EXPRESSION OF F SEX FACTOR *TRAJ* GENE

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The gene for *traJ*, the key regulatory protein involved in F plasmid gene expression, has been cloned and sequenced. Plasmids have been constructed in which *traJ* transcription is initiated from the λ PL promoter and high levels of protein expression obtained. These constructs facilitate purification of the *traJ* protein and experiments to investigate its action will be discussed.

CONVERGENT TRANSCRIPTION OF THE F PLASMID TRANSFER CONTROL GENES *FINP* AND *TRAJ*

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We have mapped the *finP* gene of the F plasmid by sequencing point mutants. The *finP* gene lies in a DNA region which is transcribed from two convergent promoters: in one direction from its own promoter and in the opposite direction from the *traJ* promoter.

The nature of the *finP* gene product and the role of convergent transcription in the control of plasmid transfer will be discussed.

GENES INVOLVED IN PHOSPHATASE SYNTHESIS IN *ASPERGILLUS NIDULANS* AND THEIR RELATIONSHIP WITH CERTAIN TRANSPORT SYSTEMS

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In *Aspergillus nidulans pac*C$^-$ mutations leading to loss of an acid phosphatase pleiotropically reduce uptake capacity for γ-amino-n-butyrate (GABA) (Arst, H. N. Jr., Bailey, C. R. and Penfold, H. A., *Arch. Microbiol.*, *125*, 153, 1980) and probably also molybdate (Arst, H. N. Jr. and Cove, D. J., *Molec. gen. Genet.*, *108*, 146, 1970). Mutations in the *pal*A,

*pal*B, *Pal*C, *pal*E, and *pal*F genes leading to loss of phosphate-repressible alkaline phosphatase as well as enhanced levels of acid phosphatase (Dorn, G., *Genet. Res.*, *6*, 13, 1965) apparently enhance GABA and molydate transport levels whereas a *pal*D$^-$ mutation, which leads to loss of phosphate-repressible alkaline phosphatase without affecting acid phosphatase, has apparently no effect on transport. *pac*C$^-$ mutations suppress *pal*A$^-$, *pal*B$^-$, *pal*C$^-$, *pal*E$^-$ and *pal*F$^-$ but not *pal*D$^-$ mutations for all pleiotropic effects (such that *pac*C$^-$ *pal*A$^-$, etc, double mutants are phenotypically indistinguishable from corresponding *pac*C$^-$ single mutants). The selection of mutations affecting the synthesis, maturation and/or secretion of phosphatases in several previously unidentified genes and their phenotypes will also be reported. As yet, the nature of the relationship between phosphatases and certain transport systems remains unclear.

A NOVEL PHOSPHATE-REPRESSIBLE PHOSPHODIESTERASE IN *ASPERGILLUS NIDULANS*

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In addition to the nitrogen metabolite-repressible cyclic nucleotide-binding phosphodiesterase–phosphomonoesterase described by G. M. Polya, A. G. Brownlee and M. J. Hynes (*J. Bacteriol.*, *124*, 693, 1975), *Aspergillus nidulans* has a phosphate-repressible phosphodiesterase with unusual substrate specificities and inhibitor sensitivities. This enzyme, which lacks phosphomonoesterase activity, is a glycoprotein which exists in multiple electrophoretically and physically separable forms. In low phosphate media, only one of these forms is appreciably secreted into the medium.

A number of the mutations described by G. Dorn (*Genet. Res.*, *6*, 13, 1965) affect levels of this phosphodiesterase. *pac*C$^-$ mutations, leading to loss of acid phosphatase, markedly reduce phosphodiesterase levels whereas *pal*A$^-$, *pal*B$^-$, *pal*C$^-$, *pal*E$^-$ and *pal*F$^-$ mutations, leading to loss of phosphate-repressible alkaline phosphatase and enhanced acid phosphatase activity, elevate phosphodiesterase levels. *pal*C$^-$mutants, which lack both acid and alkaline phosphatases, are similarly unable to exhibit phosphate-derepression of the phosphodiesterase. We have selected suppressors of *palc*A$^-$ mutations, including a mutation designated *r*D-3 which elevates both repressed and derepressed levels of acid phosphatase, alkaline phosphate and, especially, this phosphodiesterase.

THE CASE OF THE MISSING MUTANTS OR DOES *TAM*A PLAY A ROLE IN NITROGEN METABOLITE REPRESSION IN *ASPERGILLUS NIDULANS*?

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Nitrogen metabolite repression in *Aspergillus nidulans* is mediated by a positive acting regulatory gene designated *are*A (Arst, H. N. Jr and Cove, D. J., *Molec. gen. Genet.*, *126*, 111, 1973). Loss of function mutations (*are*A^r) lead to inability to utilise nitrogen sources other than ammonium whereas other, much rarer, alleles (*are*A^d) lead to nitrogen metabolite derepressed expression of one or more of the structural genes under *are*A control. J. A. Pateman and J. R. Kinghorn (in *Genetics and Physiology of Aspergillus*, eds. Smith, J. E. and Pateman, J. A., Academic Press, London, pp. 203–241, 1977) proposed that the *tam*A gene plays an equally important regulatory role in nitrogen metabolite repression in *A. nidulans* as the result of their work with "*tam*A^r-50", an "allele" leading to inability to utilise nitrogen sources other than ammonium and "*tam* A^d-1", an 'allele" leading to nitrogen metabolite derepression. Unfortunately, both "*tam*^{r}A^r-50" and "*tam*A^d-1" were subsequently lost. We have done some detective work (with a bit of science thrown in) to reconstruct Pateman and Kinghorn's work with *tam*A.

A NEAR TERMINAL PERICENTRIC INVERSION LEADS TO NITROGEN METABOLITE DEPRESSION IN *ASPERGILLUS NIDULANS*

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The mutation *xpr*D-1 was selected by B. L. Cohen (*J. gen. Microbiol.*, *71*, 293, 1972) as leading to nitrogen metabolite derepression in *Aspergillus nidulans*. It is an allele of the positive acting regulatory gene *are*A which mediates nitrogen metabolite repression (Arst, H. N. Jr. and Cove, D. J., *Molec. gen. Genet.*, *126*, 111, 1973). Here I show that *xpr*D-1 is associated with a near terminal pericentric inversion in linkage group III. The left arm break-point is between the *ad*I and *s*C genes, and the right arm break-point is between the *orn*C and *are*A genes but just centromere proximal to *are*A. One class of duplication-deficiency progeny is recovered in crosses of *xpr*D-1 strains to inversion-free strains. These progeny have two copies of the distal portion of the left arm beginning just before *s*C but lack a copy of *are*A and the region distal to it on the right arm. The viability of these duplication-deficiency progeney shows that no indispensable gene can lie distal to *are*A, suggesting proximity of *are*A to telomere. The inversion might increase expression of *are*A which, given the positive acting nature of this regulatory gene, would result in nitrogen metabolite derepression. If increased expression be the result of fusion to (or creation of) a more efficient promoter and/or ribosome binding sequence, *are*A must be transcribed towards the right arm telomere.

MAINTENANCE OF GENETIC DIVERSITY

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There appears to be more disagreement over the nature of the factors maintaining genetic diversity than is warranted by the data. Some of the different interpretations will be reviewed, with a view to establishing the extent of the common ground.

THE POSSIBILITY OF STRESS-TRIGGERED EVOLUTION

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The inadequacy of traditional evolutionary theory is becoming painfully apparent. We are faced with the necessity of explaining not only periods of rapid evolution (punctuation) but also the long periods of stasis between such punctuations. We must integrate the avalanche of new information from molecular biology, not just by adding it on to current theory but by proposing whole new theories to take it into account. Some beginning has been made by the recent proposals of "selfish" (or perhaps more properly parasitic) DNA that dwells as a passenger in the nucleus, and "molecular drive" in which various mechanisms lead to changes in the distribution and increases in the number of copies of segments of DNA.

I suggest in this talk that the well-known phenomenon of increased phenotypic variance with increased environmental stringency may in part be due to newly-induced alterations in the genome that are in fact mutations in the broadest sense. I draw an analogy with lysogenic bacteria, in which the lytic cycle can often (but not always) be triggered by a variety of quite mild environmental treatments that interfere with DNA synthesis. Such previously unsuspected mutagenesis would *not* lead to the point mutations familiar to geneticists, but would rather stimulate the excision and reinsertion of transposons. These in turn would result in mutational rearrangements more likely to affect development than would point mutations in protein products. Thus, the heat and ether treatments used by Waddington in his famous

experiments to increase phenotypic variability in *Drosophila* may in fact have produced somatic and germ-line mutations affecting development. It may have been these new alterations rather than pre-existing variability in the population that Waddington was eventually able to select. Some suggestions for testing this hypothesis are given in the talk.

GENETIC DIVERSITY AND THE NEUTRAL MUTATION THEORY

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The neutral theory of molecular evolution allows the existence of a large proportion of deleterious mutations (purifying selection) but asserts that most of nucleotide substitutions in evolution and molecular polymorphisms in natural populations are caused by neutral mutations and random genetic drift. This theory and some of its alternative theories provide predictions about the relationship between the level of protein polymorphism and population size. Examination of this relationship for many different vertebrate and invertebrate species indicates that the observed relationship is closer to the prediction from the neutral theory than that from the alternative theories when the bottleneck effect is taken into account. The patterns of nucleotide substitution and nucleotide diversity in globin and immuno-globulin genes are also in agreement with predictions from the neutral theory. Furthermore, the total amounts of DNA polymorphism in natural populations are so extensive, that the intensity of selection, if any, must be generally very small for individual pairs of alleles. Implications of these findings on morphological evolution will also be discussed.

DIVERSITY DUE TO FREQUENCY-DEPENDENT SELECTION

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Many of the ecological factors that cause death, or reduce fertility, in natural populations act in a density-dependent manner. Competition, predation, and disease are obvious examples. When these factors discriminate between genotypes it is likely that the resulting selective values will be frequency-dependent.

Many of the arguments between "neutralists" and "selectionists" are based upon the assumption that selective values are constant. The evidence of widespread frequency-dependence suggests that these arguments are misplaced.

Frequency-dependent selection can maintain large amounts of genetic variation without the practical and theoretical problems posed by heterozygous advantage on the one hand, and random genetic drift on the other.

ECOLOGICAL, POPULATIONAL AND LIFE HISTORY CHARACTERISTICS OF ALLOZYME POLYMORPHISM

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The evolutionary significance of allozyme polymorphisms in several hundreds of species, mostly in which 14 or more loci were tested, will be explored by correlating the levels of polymorphism and heterozygosity with biotic factors (ecological, populational and life history characteristics). The estimates of genetic diversity were taken from the literature and will be related to biotic profies of each species comprising ecological, populational and life history characteristics derived from direct correspondence with the individual researchers who studied the species electrophoretically. The results will be presented and discussed in an attempt to highlight the nature of allozyme polymorphisms, whether adaptive, neutral, or both.

GENETIC DIVERSITY IN SELECTION MODELS

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The predictions concerning genetic diversity of selection–mutation–migration models are presented for finite as well as infinite populations. The robust nature of selection models and the approach to neutral limit are discussed. The effect of environmental fluctuations, and frequency and density dependent selection are considered. Results for multi-locus systems are discussed. The implication of these results on evolution is discussed.

MOBILIZATION AND TRANSFER OF SYMBIOTIC PLASMIDS IN *RHIZOBIUM TRIFOLII*

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Symbiotic and cryptic plasmids from *Rhizobium trifolii* have been labelled by the transposon Tn5 mob, which confers on them the ability to be mobilized and transferred to other *Rhizobium* strains and *Agrobacterium*. Sym plasmid transfer to non-nodulating *Rhizobium* restores their symbiotic proficiency. Preliminary incompatibility data between the sym plasmids and others will be discussed.

CLONING AND EXPRESSION OF *BACILLUS LICHENIFORMIS* ALPHA AMYLASE GENES IN *BACILLUS SUBTILIS*

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Bacillus species secrete extracellular enzymes in response to nutritional deprivation. These enzymes include nucleases, proteases and polysaccharases and are of considerable commercial importance. At the genetic level the most extensively characterised of the exoenzymes is the alpha-amylase system of *B. subtilis*. Although a number of loci are known to be involved in the regulation of amylase expression, the molecular basis of this regulation remains obscure.

In order to investigate the molecular control signals governing amylase gene expression we have isolated two alpha-amylase genes from two *B. licheniformis* species by molecular cloning in *B. subtilits*. These genes were shot-gun cloned into *B. subtilis* using the vector pBD64 and the method of homologous plasmid assist (Gryczan, T. *et al.*, *Molec. gen. Genet.*, *177*, 459–467, 1980). The amylase genes code for thermostable enzymes of slightly, but measurably different, temperature optima. Detailed restriction endonuclease cleavage maps have been constructed and the location and direction of transcription of the amylase genes determined. Nucleotide sequencing is underway to define the regulatory regions preceding the amylase structural gene.

GENES FOR GALACTOSE UTILISATION IN *STREPTOMYCES COELICOLOR* A3(2)

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Spontaneous 2-deoxygalactose-resistant mutants were isolated in *Streptomyces coelicolor* A3(2). Most proved to be Gal^{-1} and their reversion frequenceis were measured. Attempts to clone the *gal* gene(s) by complementation of these mutations will be discussed.

THE INFLUENCE OF HOST ORGANISM ON THE STABILITY OF R-PRIME DERIVATIVES R68·45

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J. H. SLATER

Department of Applied Biology, UWIST, Cardiff

Transfer of R' plasmids carrying the Fraction I dehalogenase gene of *Pseudomonas putida* PP3 to a range of host organisms resulted in the precise excision of inserted DNA. The possibility of a plasmid-borne integrity function will be discussed.

STABILITY OF THE R-PRIME pUU2 GROWN IN CONTINUOUS CULTURE UNDER PHOSPHATE LIMITATION

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pUU2 is an R' derivative of R68·44 carrying the Fraction I dehalogenase gene of *Pseudomonas putida* PP3. *Pseudomonas putida* PaW 340 containing pUU2 was grown in a chemostat on 2-monochloropropionic acid as sole carbon and energy source under phosphate limitation. Derivatives of the original strain were isolated from the chemostat, these contained plasmids which exhibited a range of excision events

STUDIES ON LINKAGE OF RESTRICTION SITE POLYMORPHISMS TO DUCHENNE, BECKER AND MYOTONIC MUSCULAR DYSTROPHIES

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Restriction fragment length polymorphisms, detected by Southern blotting and hybridisation to cloned fragments of human X and 19 chromosomes, are being used as linkage markers for locating and identifying the genes responsible for Duchenne, Becker and myotonic dystrophies.

TOWARDS THE CLONING OF THE CALF PROCHYMOSIN GENE

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An Foras Taluntais, Moorepark, Fermoy, Ireland

F. OLLINGTON

Biocon Limited, Kilnagleary, Carrigaline, Co. Cork, Ireland

The calf prochymosin (rennin) gene is of importance commercially. We have initiated a programme to clone this gene, using recombinant DNA methods, initially in *E. coli* and ultimately in *B. subtilis*. To date, we have carefully analysed the calf stomach mRNA, the pattern of prochymosin expression in different calves and the antisera which we have prepared against the purified prochymosin protein. At present, cloning experiments are underway using ds cDNA. The progress made with these experiments will be reported. Two forms of this gene have previously been cloned (1, 2).

RUNAWAY REPLICATION DERIVATIVES OF PLASMID R1 AS TOOLS IN GENETIC ENGINEERING

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S. LIGHT

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S. MOLIN

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We describe here new temperature-dependent runaway replication derivatives of plasmid R1, whose construction was based on our current knowledge of the plasmid's replication control system. These new plasmids have certain advantages over the original runaway plasmids as vectors for cloning foreign genes in *E. coli*, particularly if the cloned gene product is likely to be detrimental to host viability.

TIMA AS A MECHANISM OF MOLECULAR EVOLUTION

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A talk on template-induced molecular assembly (TIMA) mechanisms and their implications will be based on the following papers, and on other recently completed work on related topics.

Wassermann, G. D. (1982) TIMA Part 1. TIMA as a Paradigm for the evolution of Molecular Complementarities and Macromolecules. *J. theor. Biol.*, *96*, 77–86.

Wassermann, G. D. (1982) TIMA Part 2. TIMA-based instructive Evolution of Macromolecules and Organs and Structures. *J. theor. Biol.*, *99*, 609–628.

PROTEINS SPECIFIED BY THE GENETICALLY SILENT TERMINUS REGION OF THE *E. COLI* CHROMOSOME

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We have cloned approximately 20 kb of *E. coli* E12 DNA from the chromosomal replication terminus region, *ter*C. This region is known to contain only one other genetic locus, *trg*. Protein synthesis directed by our recombinant plasmids in a mini-cell system, has shown this genetically "silent" region to code for several polypeptides of apparent molecular weights ranging from below 20 KD to over 100 KD.

FORMATION OF INTEGENERIC YEAST HYBRIDS BY PROTOPLAST FUSION

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Hybrids have been made between *Yarrowia lipolytica* and two other yeasts, *Kluyveromyces lactis* and *Saccharomyces diastaticus*, by means of protoplast fusion. Protoplasts were prepared from auxotrophic mutants of these organisms and the two strains to be fused co-centrifuged and resuspended in PEG-Ca^{2+}. The fusion mixture was plated in minimal medium to select for prototrophic fusants, which arose at a frequency between 2×10^{-5} and 5×10^{-4}. The frequency of reversion of the auxotrophic markers employed was $<1\times10^{-8}$.

The *Y. lipolytica* × *K. lactis* fusants have a morphology intermediate between that of the two parent strains. Fluorescence microscopy demonstrated that the majority of fusant cells were uninucleate although a small proportion of cells contained 2–8 nuclei. The cross between *Y. lipolytica* (met$^-$, white) and *S. diastaticus* (ade$^-$, red) yielded white prototrophic fusants restreaking these colonies gave a range of colony types from white through to various shades of pink to red. This variation cannot be attributed to the breakdown of an unstable heterokaryon since the majority of fusants cells were uninucleate, with a diploid DNA constitution.

The physiological and molecular biological characterisation of the fusants is in progress. The fusants have slower growth rates than the parental species and exhibit carbon source assimiliation patterns intermediate between those of the parents. The relative contribution of the two parental genomes to the fusants' phenotype will be discussed.

PURIFICATION AND CHARACTERISATION OF A DOUBLE-STRANDED RIBONUCLEASE FROM *SACCHAROMYCES CEREVISIAE*

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Most laboratory strains of *Saccharomyces cerevisiae* contain at least one species of double-stranded RNA (dsRNA). We have demonstrated previously that dsRNA is degraded when a prototrophic strain is starved for nitrogen. We now report the purification of a dsRNase from yeast. The enzyme has been purified greater than 90-fold from stationary phase cells of A364A. Purification involved differential centrifugation, DEAE-cellulose chromatography and phosphocellulose chromatography. The enzyme has an apparent molecular weight of 27,000 in SDS-polyacrylamide gels. It shows a high specificity for dsRNA. Its physical and biochemical properties have been compared with those of RNase 111 from *E. coli*. A number of differences have been observed, the most striking of which is that the yeast enzyme is markedly inhibited by Mg^{2+} or Mn^{2+} whilst these cations stimulate RNase 111 activity. Changes in the specific activity of the enzyme through the yeast batch growth curve and during nitrogen starvation have been studied. The possible physiological role of this enzyme will be discussed.

ISOLATION AND CHARACTERISATION OF THE *SPOIIA-TYRA* REGION *B. SUBTILIS* CHROMOSOME

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pC12 and pRC15 contain *B. subtilis* DNA from the *spoIIA-tyrA* region and were isolated from a "shotgun" cloning experiment and a cosmid bank respectively. Both are unstable under certain conditions and contain DNA encoding for several loci involved with differentiation. Attempts to subclone these genes and to characterise the instabilities are being made.

A GENETIC STUDY OF *ERWINIA CAROTOVORA*

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The bacterial phytopathogen *Erwinia carotovora* subsp. *carotovora* (*Ecc*) causes soft-rots of the parenchymatous tissue of several plant species, with rots of stored potatoes being of particular importance in the UK. *E. carotovora* subsp. *atroseptica* is host specific to potatoes where it is the causal agent of blackleg. A genetic approach to the study of this bacterium was undetaken because although the biochemistry of the infectious process is now quite well understood, little is known of the genetic basis of pathogenicity. After screening several strains of *Ecc* and *Eca* for the presence of plasmids, it was found that they were not common to all strains. Consequently research was centred on genetic manipulation of the bacterial chromosome. With the establishment of two conjugational gene transfer systems in *Ecc* SCRI193, it was possible to construct a circular linkage map of the chromosme, which consists of 17 ordered genes. Current research is being directed at the development of an efficient method of transposon mutagenesis and an *in vitro* cloning system.