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IDENTIFYING PROTEIN-CODING SEQUENCES: THE COMPLETE DNA SEQUENCE OF BACTERIOPHAGE T7 USED AS A TEST OF THE RNY HYPOTHESIS

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Protein-coding DNA sequences have an excess of RNY (purine, any base, pyrimidine) triplets in the reading frame. Pieczenik (*PNAS, 77*, 3539–3543, 1980) predicted such a feature for *E. coli* genes, based on a hypothesis of a 5 base pair tRNA-mRNA interaction. Shepherd (*Cold Spring Harbor Symp., 46*, 1099–1108, 1982) has documented this excess in sequences from a wide variety of organisms (though explaining it as a relic of a commaless code), and has proposed that the RNY bias might be used to identify protein-coding regions. The determination of the complete (39936 bp) DNA sequence of bacteriophage T7 (Dunn and Studier, *J. Mol. Biol. 166*, 477–535, 1983) presents an excellent opportunity to test the RNY hypothesis over the entire genome of a particularly well genetically characterised organism. Dunn and Studier have proposed 50 essentially nonoverlapping genes (occupying 91 per cent of the total sequence), for 38 of which there exists strong biochemical and genetic evidence.

Analysis reveals a large excess of RNY codons in the whole genome of T7, not simply explicable in terms of amino acid bias in the proteins encoded. In 47 of the 50 genes the reading frame is mutationally nearer a perfect RNY repeat than either of the noncoding registers. Explanations can be offered for all 3 exceptions. Systematically scanning the T7 sequence for open reading frames with the lowest mutational distance from RNY produces expected coding regions in very good agreement with those observed.

SPECIFIC SUPPRESSION OF MUTATIONS IN THE PHAGE T1 DNA-ARREST GENES BY THE INTERNAL SUPPRESSOR MUTATION *DAS*

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T1 genes 3·5 and 4 are two of the four genes known to be essential for phage DNA replication. Amber mutations in either gene exhibit a complex phenotype under non-permissive conditions which includes: premature cessation of DNA synthesis (DNA arrest), inability to form concatemeric DNA intermediates, reduced genetic recombination, reduced levels of the T1-coded exonuclease and reduced viable phage production. We have identified a new class of T1 mutants which suppress the lethal effects specifically of gene 3·5 and 4 amber mutations. These internal suppressor mutations are called *das* (*d*na *a*rrest *s*uppressor). When coupled with amber mutations *am* 23 (gene 4) or *am* 201 (gene 3·5), the *das* mutation leads to the partial restoration of concatemer formation, late DNA synthesis and viable phage production. For example, the burst sizes of *am* 23·*das* and *am* 201·*das* in non-permissive cells can be more than 20 times higher than *am* 23·*das*$^+$ and *am* 201·*das*$^+$.

Suppression by *das* does not occur by restoration of the synthesis of the normal 3·5 and 4 gene products. Suppression by *das* is temperature-sensitive giving larger bursts at 30° than at 37°. The triple mutant *am* 23·*am* 201·*das* is more temperature-sensitive than either *am* 23·*das*

or *am* 201·*das*. This may indicate a molecular interaction between the *das* protein and either the gene 3·5 product (in *am* 23·*das*) or the gene 4 product (in *am* 201·*das*) which leads to increased thermal stability compared with that of the *das* protein alone (in *am* 23·*am* 201·*das*).

INTERACTIONS OF PHAGE T1 DNA WITH THE MEMBRANE OF THE *E. COLI* HOST

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T1 DNA replication occurs via the formation of concatemeric intermediates 3–4 genome equivalents long from which "headfuls" of DNA are processively excised for packaging into phage heads. The replication process has been analysed in terms of the relationship of the phage DNA with the host cell membrane by the M-band technique which allows the isolation of DNA-membrane complexes. Our results with ^{3}H-TdR-labelled T1 DNA show that both parental and newly-synthesised DNA form complexes with the cell membrane.

Parental T1 DNA has not been observed to form concatemers however, it does remain membrane-attached until 6–8 min post-infection when it is released along with newly-synthesised DNA. This release is blocked by mutations in T1 genes affecting head formation and DNA synthesis. Formation of parental DNA-membrane complexes is blocked by cyanide but not by chloramphenicol suggesting that phage-coded functions are not required.

In wild type infections 50 per cent of newly-synthesised T1 DNA remains membrane-bound when ^{3}H-TdR is present continuously. After short ^{3}H-TdR pulses given early during infection 80–90 per cent of the label is membrane attached. Regardless of the time of the pulse the label detaches from the membrane 6 min later. Newly-synthesised DNA is not released in cells infected with T1 mutants defective in head formation and DNA synthesis functions. Tail mutant infections behave as wild type, whereas mutations in the DNA-arrest/recombination genes (3·5 and 4) show a gradual and incomplete release pattern.

MOLECULAR STUDIES OF THE *NIAD* GENE OF *ASPERGILLUS NIDULANS*

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The minimmum size of the NADPH nitrate reductase structural gene from *Aspergillus nidulans* can be estimated from the molecular weight of the subunit monomer of this enzyme. However, various values of sub-unit size have been reported and on average these suggest a structural gene size of at least 1·5 kb. Our results show that these are underestimates and that the minimum size of the nitrate reductase structural gene is approximately 2·5 kb.

LARGE SPONTANEOUS DUPLICATIONS IN *ASPERGILLUS NIDULANS*: THE INFLUENCE OF A RECIPROCAL TRANSLOCATION

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A new leaky isoleucine-requiring mutant (0·3 per cent recombination with *ileA*1; IIR) has been used to select strains with a duplicate chromosome segment. Stunted colonies on isoleucine-free medium gave more vigorous "revertant" sectors of which c. 13 per cent were shown, by various criteria, to have a duplication of the leaky allele with a large segment of IIR (cf. Sexton and Roper, *J. Gen. Microbiol.*, in press).

Presence in the parent of the reciprocal translocation T1(VI–VII) affected genomic location of the newly-generated duplicate segments. In 11 strains sufficiently stable for analysis, all

had the duplication linked with the T1(VI–VII) complex; further analyses of six of these have shown linkage with the element of the complex associated with chromosome VI centromere. In contrast, duplicate segments in 6 analysable strains derived from the translocation-free parent had locations on chromosomes I, II, VI (2 strains), VII and VIII.

Mitotic crossing over (with appropriate chromatid segregation) eliminates the transposed duplicate segment in duplication diploids and results in extreme mitotic instability (Case and Roper, *J. Gen. Microbiol.*, *124*, 9–16, 1981). Diploids with T1(VI–VII)-attached duplications were unstable when homozygous for T1(VI–VII) but, when heterozygous for the translocation, were more stable than the respective duplication haploid; this relative stability probably resulted from the absence of duplication-eliminating mitotic crossing over. Use of appropriate heterozygous translocations may facilitate stabilisation of duplication diploids for the study of gene dosage and other effects.

INVOLVEMENT OF THE *E. COLI RECN* GENE IN INDUCIBLE DNA DOUBLE-STRAND BREAK REPAIR

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Mutation of *recN* leads to increased sensitivity to mitomycin C and ionizing radiation in a wild-type genetic background, and confers sensitivity to ultra-violet (UV) light and a recombination deficiency in *recBC sbcB* mutants (R.G. Lloyd *et al*, *Molec. Gen. Genet.*, *190*, 162–167, 1983). The presented results demonstrate that *recN* mutants are unable to repair DNA double-strand breaks. The repair of double-strand breaks requires the presence of a homologous duplex and a functional *recA* product, and is enhanced by prior irradiation of cells with UV light, suggesting that it is one of the inducible (SOS) repair activities regulated by *lexA* (F. Kraisin and F. Hutchinson, *J. Molec. Biol.*, *116*, 81–98, 1977; *PNAS*, *78*, 3450–3453, 1981; J. W. Little and D. W. Mount, *Cell*, *29*, 11–22, 1982). Expression of the *recN* gene is induced by damage to DNA and is regulated by the *lexA* repressor. It would therefore appear that *recN* is involved in an inducible recombination process specifically for the repair of double-strand lesions, and may well be the basis of the observation that induced radioresistance is an aspect of SOS repair (E. C. Pollard *et al.*, *Mol. Gene. Genet.*, *184*, 421–429, 1981).

A *lexA*(Ind$^-$) mutation reduces the expression of most SOS genes by no more than about two-fold. However expression of *recN* is reduced approximately 20- and 40-fold in wild-type and *recBC sbcB* mutants respectively. The *lexA*(Ind$^-$) mutation also blocks recombination in *recBC sbcB* mutants (R. G. Lloyd and A. D. G. Thomas, *Mol. Gen. Genet.*, *190*, 156–161, 1983). We propose that this recombination deficiency is due to severely reduced synthesis of the *recN* gene limiting inducible recombination.

THE USE OF *SALMONELLA TYPHIMURIUM* STRAINS TA 98NR AND TA 98/1,8-DNP$_6$ IN THE ASSESSMENT OF THE MUTAGENIC ACTIVITY OF COMPLEX ENVIRONMENTAL MIXTURES

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The strains TA 98NR and TA 98/1,8-DNP$_6$ are insensitive to the mutagenic activity of certain nitro-substituted aromatic hydrocarbons and have been used to estimate the contribution of such compounds to the overall mutagenic activity of complex mixtures. Work carried out in our laboratories has shown that the strain TA 98/1,8-DNP$_6$ is also insensitive to other chemical classes, *i.e.*, non-nitrated aromatic agents. In view of these findings the interpretation of results obtained for environmental mixtures using this strain is discussed. The role of bacterial enzymes in the conversion of materials to mutagenic species is also discussed.

EFFECT OF EXPOSURE TIME ON THE MUTAGENIC POTENCY OF THE ANTITUMOUR AGENT ICRF 159 IN L5178Y CELLS *IN VITRO*

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The antitumour agent ICRF 159 (Razoxane) is capable of inducing cytotoxicity and an increase in mutation frequency at the thymidine kinase locus in mouse lymphoma L5178Y cells *in vitro*. However, the dose responses obtained in both cases are non-linear when exposure is limited to 4 hours, and both the cytotoxic potency and the mutagenic potency can be shown to decrease with increasing drug concentration under these conditions. When the exposure time is extended to 24 hours, ICRF 159 induces a dose-dependent linear decrease in cell survival with a concomitant dose-dependent linear increase in mutation frequency at the thymidine kinase locus. Under these conditions the mutagenic potency and cytotoxic potency of the agent are independent of drug concentration.

Since the mutagenic potency and other agents in these cells can be shown to be essentially unaffected by the duration of exposure it is suggested that the induction of mutagenic lesions by ICRF 159 may be subject to a cell cycle specificity similar to that which has previously been demonstrated for its cytotoxic mode of action.

The results presented also illustrate a potential drawback when attempting to compare the activity of agents in this assay system on the basis of mutagenic potency.

MOLECULAR EVOLUTION OF RICE SPECIES

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The evolutionary relationships of rice species have been investigated using two distinct approaches. Isoelectric focussing has been used to analyse inter- and intra-specific differences in Fraction I protein; blot hybridisation techniques have been employed to study changes that have occurred between rice genomes in the organisation and amounts of satellite and interspersed repeated DNA sequences. The results from these two approaches produce a clear and coherent picture of rice evolution, which will be discussed with reference to previous theories.

THE DISTRIBUTION OF SPECIFIC REPEATED DNA SEQUENCES ALONG THE CHROMOSOMES OF *ALLIUM* SPECIES

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In situ hybridisation techniques have been used to analyse the chromosomal distribution of sequences homologous to three different cloned repeated sequences. The results will be described and discussed with reference to models of repeated sequence amplification and dispersal through the genome.

THE ISOLATION OF mRNA FROM WHITE CLOVER LEAF TISSUE OF KNOWN GENOTYPE AT THE LOCI CONTROLLING CYANOGENESIS

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Since white clover is a perennial species and is easily vegetatively propagated, it is possible to produce large quantities of leaf material from genetically characterised plants. A technique for the isolation of mRNA from developing leaf tissue has been developed. The mRNA

produced gives a 25–30 fold stimulation of translation activity in a wheat germ translation system and is a good template for cDNA synthesis. Comparisons of linamarase mRNA were made between white clover plants with different alleles at the *Li* locus.

MUTAGENIC EFFECTS OF LEAD IN *SCHIZOSACCHAROMYCES POMBE*

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A variety of lead associated hazards to human health have been described. High levels in children have sometimes resulted in death due to neurological toxicity, while several biochemical aberrations, reduced IQ and chromosomal damage have been attributed to lower levels. We have examined the possible mutagenic effects of lead by monitoring induced mutation frequencies in continuous cultures of the fission yeast, *Schizosaccharomyces pombe*. As this system is designed to detect chronic levels of mutagenic activity, toxic effects, which have caused problems in previous studies into the mutagenicity of lead, are reduced. The implications of our findings will be discussed.

THE INFLUENCE OF SV40 AND EPSTEIN-BARR VIRUSES ON THE INDUCTION BY γ-IRRADIATION OF 6-THIOGUANINE RESISTANT MUTANTS IN ATAXIA-TELANGIECTASIA CELLS

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Untransformed skin derived fibroblasts from ataxia-telangiectasia (A–T) patients are hypersensitive to the lethal action of γ-irradiation but are hypomutable for the induction of 6-thioguanine resistance. These results suggest that the cells may lack error-prone repair processes which confer resistance at the price of mutation in normal cells. In contrast thioguanine resistant mutants are induced in a dose dependent fashion by γ-irradiation in the A–T cell strain AT5BI which has been transformed by SV40 virus and are also induced in lymphoblastoid line ATL6. Concomittantly, the survival of SV40 transformed cells is enhanced.

The modulation of mutability together with the effect on survival suggests that viral transformation may switch on error-prone repair processes in these cells.

THE CLONING AND EXPRESSION OF THE *MUT*H AND *MUT*S GENES OF *ESCHERICHIA COLI*

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The *mut*H gene has been cloned in a λ factor, λ 540, pBR322 and pP1c236. The only polypeptide associated with a functional *mut*H gene when λ *mut*H infected heavily irradiated cells was found in the cell envelope fraction; it had an apparent size of 24,000 daltons. But *mut*H cloned in pBR322 and pP1c236 gave as the major product, in the "maxi-cell" method, a 27,000 dalton polypeptide, again mainly associated with the cell envelope. However, in the second case the 24,000 dalton product was also observed. It will be argued that the larger polypeptide is the initial *mut*H gene product and that it is processed to yield the smaller.

The *mit*S gene has been cloned on pBR322. Its gene product as determined by the maxi-cell method is a 93,000 polypeptide which also shows a strong, if less marked, affinity for the cell envelope.

FACTORS AFFECTING MUTANT YIELD IN *E. COLI* IN LIQUID SUSPENSION ASSAYS

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The liquid suspension assay (Green and Muriel, *Mutation Res.*, *38*, 3–32, 1976) allows the determination of several genetic endpoints in the same culture. However, it has proved less sensitive than plate incorporation tests in detecting mutagens. We have found that the sensitivity of the former assay is increased, especially where slightly toxic concentrations of mutagens are used, by allowing the mutagen-exposed cells an overnight-growth period in nutrient broth before plating on selective agars. For example using *E. coli* WP2 *uvrA*(pKM101), the number of trp^+ revertants per plate induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; 50 μg/ml) methylmethane sulphonate (2·6 mg/ml) or 4-nitroquinoline-N-oxide (NQQ; 5 μg/ml) was 7, 30 and 1332, respectively, before, and 874, 849, and 2292 respectively, after the overnight growth period. When mutation to resistance to certain antibiotics was used as a genetic end point, mutagenicity was only detected after the overnight growth period.

Exposure of cells to some mutagens, *e.g.*, MNNG and NQQ, under non-growing conditions (minimal salt medium) induced more mutants per plate than when the cells were exposed in a growth medium (minimal salts plus glucose). A mutagenic effect can also be modified by the presence of absence of dimethylsulphoxide. In particular, the number of *E. coli* trp^+ revertants induced by MNNG (5 to 50 μg/ml) was increased approximately 8 fold in the presence of dimethylsulphoxide.

We thank MAFF for financial support.

ESCHERICHIA COLI CULTIVATED IN LOW CONCENTRATIONS OF DICHLORVOS ARE RESISTANT TO THE MUTAGENIC EFFECTS OF SUBSEQUENT N-METHYL-N'-NITRO-N-NITROSOGUANIDINE TREATMENT

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We have studied the effect of cultivating *E. coli* WP2 in low concentrations of the insecticide Dichlorvos (DCV) on mutation-induction and cytotoxicity after treatment with various mutagens. *E. coli* cultures, cultivated in known concentrations of DCV (0–22 μg/ml), were challenged with DCV (3000 μg/ml), 4-nitro-quinoline-N-oxide (4NQO; 3 μg/ml) or N-methyl-N-nitro-N-nitrosoguanidine (MNNG; 10 μg/ml). Cultivation in DCV had no effect on the lethal or mutagenic effects of a subsequent challenge with either itself or 4NQO. However, cultivation in DCV did bring about a linear decrease in the mutagenic (but not cytoxic) effects of a subsequent MNNG challenge. Cultivation in a low concentrations of either 4NQO (0–0.6 μg/ml) or MNNG (0–1.0 μg/ml) had no effect on a subsequent DCV challenge.

DCV treatment induces a cell function capable of repairing mutagenic (but not lethal) DNA lesions produced by MNNG, but not those produced by 4NQO or DCV itself.

We thank the UK Ministry of Agriculture, Fisheries and Food for financial aid.

DETECTION OF SOMATIC MUTATION IN THE RETINAL PIGMENTED EPITHELIUM BY CHANGES IN GRANULE SIZE

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Beige (*bg*) is a cell-autonomous autosomal recessive coat colour mutant in the mouse that reduces pigment intensity by increasing granule size. The giant granules are highly conspicuous in the melanocytes of the retinal pigmented epithelium (RPE). By exploiting this drastic change in granule size it is possible to detect forward mutational events in the single layer of hexagonal

cells comprising the RPE. In a pilot study, heterozygotes for *bg* were exposed to 2.0 Gy X-irradiation at different stages of foetal development. 3 days after birth, before choroidal pigmentation is fully established, the mice were killed and the eyes prepared for microscopic observation as flattened whole mounts. Clones of retinal melanocytes containing large granules of *bg* phenotype were observed amongst those containing normal pigmentation. The number of presumptive clones per eye and the number of mutant cells per clone declined with increasing foetal age but frequencies were greater than in untreated controls. Pigment granules were extracted from the adult eyes of beige and wild type mice. Analysis with Coulter counter or Quantimet revealed marked differences in size distribution, although the presence of choroidal granules was a complicating factor. Elimination of this by the use of suitable mutants should allow automation of this method of *in vivo* somatic mutation detection.

AZA-CYTIDINE INDUCES REVERSION IN THYMIDINE KINASE DEFICIENT MOUSE CELLS

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There has always been argument as to the origin of stable variants of animal cell lines. For some variants considerable evidence now exists showing that they are the result of changes in the base sequence of nuclear DNA (A. E. Simon and M. W. Taylor, *Proc. Natl. Acad. Sci., USA, 83*, 810, 1983). Other variants are claimed to arise from regulatory epigenetic events analogous to those occurring during differentiation. Until recently, no evidence had been produced for any mechanism which could be responsible for such epigenetic events. The position has now changed since several agents including 5-aza-cytidine, butyric acid and ethionine have been shown to cause induction of thymidine kinase in stable variants in Chinese hamster cells deficient in this enzyme (M. Harris, *Cell, 29*, 483, 1982).

We have examined the effect of 5-aza-cytidine on thymidine kinase deficient variants of a mouse cell line, Friend erythroleukaemia cells. A 24 hour exposure to concentrations of 5-aza-cytidine ranging from 0·6–2·4 μg/ml induces stable expression of thymidine kinase, as measured by HAT selection, in up to 2 per cent of the surviving cells. Since 5-aza-cytidine is a potent hypomethylating agent these results suggest a role for methylation of DNA in the loss of thymidine kinase activity in these variants.

IN VITRO METABOLISM OF 4-CYANO-N,N-DIMETHYL-[^{14}C]ANILINE (CDA] BY RAT, MOUSE, CHICKEN AND INDUCED RAT "S9" FRACTIONS

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CDA has been investigated in the second UKEMS collaborative study using a variety of test systems. Common to many of the systems were the "S9" fractions used. The quantitative and qualitative metabolism of CDA by the S9 fractions from rat, mouse, chicken and induced rat has been investigated.

Under conditions analogous to those used in the tests it was found that the capacity to metabolise CDA lay in the order: induced rat > mouse > chicken > non-induced rat. In each case, N-demethylation was the major metabolic route. There were small amounts of more polar metabolites produced (<10 per cent) but none of these corresponded to any of the known *in vivo* metabolites from the rat or the mouse.

MOLECULAR ANALYSIS OF ACTIN GENES ISOLATED FROM TOBACCO AND POTATO

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Correct developmental expression of genes transduced into the germ-lines of eukaryotes have been shown to be governed by lengths of cis-acting sequences (*Cell, 34*, 37–45, 47–54,

59–73). The *Agrobacterium* Ti-plasmid based transformation system makes such an analysis possible in dicotyledonous plants. The first step in the detection of cis-acting gene regulatory sequences involves isolation of specific genes along with flanking sequences from the plant genome. Several genomic sequences coding for the cytoskeletal protein actin have been isolated from bacteriophage Lambda derived libraries of plant DNA. What does the large number of these genes mean? The observed restriction pattern differences around these genes may reflect differences in the cis-acting control elements that may possibly regulate each actin gene differently. Differences in the coding region of the genes may indicate possible subcellular and tissue specific actin types (*J. Mol. Biol.*, *163*, 533–551). The utilisation of presumptive control regions in chimeric gene constructions could help identify:

(i) Cis-acting gene regulatory sequences in the plant genome,
(ii) Tissue specific forms of members of the gene family,
(iii) Involvement of cis-acting sequences in controlling tissue specific expression of genes.

THYMINELESS MUTAGENESIS IN THE cI GENE OF LAMBDA PROPHAGE

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Thymine starvation may be weakly mutagenic (Witkin and Wermundsen, *CSH Symp.*, *43*, 881–886, 1978) or strongly mutagenic (Bresler *et al*, *Mutation Res.*, *19*, 281–293, 1972). The extent of mutagenesis observed may depend on the mutation assay system and on the lethality of the treatment. We have used a forward mutation system in which bacteria unable to form colonies can still express mutations. Thymine auxotrophs lysogenic for phage λ*ind* are starved of thymine and mutations subsequently expressed during growth in complete medium. cI gene mutations in λ prophage block repressor synthesis and produce a single burst of cI mutant phages (Blattner *et al.*, *Virology*, *62*, 458–471, 1974). The cI mutants can be easily multiplied and characterised.

In *E. coli* GY158, maximum levels of mutagenesis (6×10^{-8}/base pair) are found after 120 m thymine starvation. Mutagenesis extends beyond the period of 70 m required for maximum SOS induction and is unlikely to be an "untargeted" SOS effect.

Two independent *recA* strains show substantial thymineless mutagenesis confirming that the mechanism is not entirely SOS-mediated. Experiments in other genetic backgrounds will be described.

Initial screening of the mutants has provided some evidence for the presence of insertions.

EFFECTS OF CAFFEINE TREATMENT ON MITOMYCIN C-INDUCED CYTOGENETIC DAMAGE IN WILD-TYPE AND THYMIDINE KINASE-DEFICIENT FRIEND LEUKAEMIA CELLS

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Clone 707 of the Friend murine leukaemia cell line has a high spontaneous mutation frequency at the thymidine kinase locus (P. G. McKenna and I. Hickey, *Mutation Res.*, *80*, 187, 1981). Deficiency of the enzyme in Friend cells leads to greatly increased sensitivity to cell-killing, mutagenesis, and the induction of cytogenetic damage by mutagenic agents (P. G. McKenna and A. A. Yasseen, *Genet. Res.*, *40*, 207, 1982); A. A. Yasseen and P. G. McKenna, *Heredity*, *50*, 207, 1983) suggesting a role for the enzyme in DNA repair processes.

The effects of a non-toxic dose of caffeine (150 mg/ml) on the induction of cytogenetical aberrations following mitomycin C (MMC) treatment was measured in clone 707 and a thymidine kinase-deficient subclone, 707BUF. Four doses of MMC were used (0·025, 0·05, 0·075 and 0·10 μg/ml). Mutagen treatment was for 16 hours and with each dose caffeine treatment was as follows, (a) not present, (b) present during mutagen treatment and for 12 hours post-treatment, (c) present for 12 hours post-mutagen treatment.

There was a significantly greater frequency of cytogenetical aberrations, at all mutagen doses alone and coupled with all caffeine treatments, in subclone 707BUF relative to wild-type cells. Caffeine treatments enhanced the frequency of cytogenetic damage induced by MMC.

This effect of caffeine was much more pronounced in subclone 707BUF than in wild-type cells.

Thymidine kinase deficient cells may be highly mutagen-sensitive through an imbalance in nucleotide pools rendering excision repair error-prone. It is suggested that caffeine may enhance this sensitivity by inhibiting post-replication repair processes so that thymidine kinase deficient cells in the presence of caffeine can only repair DNA damage through an error-prone system.

SPECIES AND SEX DIFFERENCES IN THE CLASTOGENIC RESONSE TO CYCLOPHOSPHAMIDE

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Cyclophosphamide (CP) is one of the most commonly used antineoplastic agents. It has widespread use in the treatment of Burkitt's lymphoma, multiple myeloma and ovarian carcinoma. It is also used in combination with other therapeutic drugs against a variety of tumours. CP is mutagenic, teratogenic and carcinogenic in a number of animal species. For its effects, CP requires initial bioactivation via microsomal mixed function oxygenases (cytochrome P-450 system) before the ultimate active metabolite, phosphoramide mustard, is generated. This alkylating species then reacts at a cellular level to produce the genetic lesions detectable as chromosomal damage.

CP was evaluated in a series of experiments for its clastogenic potential in both sexes of man, Chinese hamster, CD-1 mouse and CD rat. CP was clastogenic in all species studied.

Sex differences in the magnitude of aberration induction were found, particularly in man and chinese hamster. In these species, exchange configurations and multiple aberrations were more frequent in females than in males. In CD-1 mouse, CP was nearly twice as effective at inducing micronuclei in polychromatic erythrocytes in females compared to males after a double dose of the agent. In CD rat, however, only the frequency of multiple aberrations was increased in females, over males, after a single dose of CP.

VIDEOS OF CLAMP CELL FORMATION AND CONJUGATE DIVISION IN *SCHIZOPHYLLUM COMMUNE* AND *COPRINUS CINEREUS*

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The genetic control of clamp cell formation and conjugate division in *S. commune* and *C. cinereus* is well known. Here we present a video of these processes as seen in living material under high magnification phase optics. You've read it in the textbooks now see the film!

MUTAGENICITY OF SEVEN ANTI-CANCER DRUGS IN *DROSOPHILA* AND CHINESE HAMSTER V79 CELLS

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Data on the ability of certain anti-cancer drugs to induce sex-linked recessive lethal mutations in *Drosophila* and mutations conferring 6-thioguanine resistance in cultivated mammalian cells, in the presence or absence of rat hepatocytes, will be presented. The drugs include adriamycin, cyclophosphamide, cytosine arabinoside, 6-mercaptopurine, methotrexate, prednisolone and vincristine.

STUDIES ON THE REDUCTIVE ACTIVATION OF DINITRO-TOLUENE BY COMPONENTS OF THE INTESTINAL MICROFLORA

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The microflora of the gastro-intestinal tract is known to be important in the metabolism and activation of various classes of genotoxic agents. Activation of genotoxicants by gut microflora frequently involves metabolic reactions which do not occur elsewhere in the body. Consequently, chemicals activated by gut bacteria may not be detected in short-term mutagenicity tests using standard metabolising systems from organ homogenates.

The hepato carcinogen 2,4-dinitrotoluene (DNT, induces UDS in the livers of rats given this compound orally, but not in animals lacking a constitutive gut microflora (mirsalis and Butterworth, *Nature*, 295, 322, 1982), suggesting that the compound needs to be activated in the small intesting prior to absorption.

DNT is mutagenic to *Salmonella typhimurium* TA98 and TA100 due to reductive activation by these tester strains. In order to identify the intestinal bacteria responsible, and to further study the mechanism of activation of DNT, the chemical has been anaerobically pre-incubated with cultures of microflora from different regions of the rabbit gastrointestinal tract. Metabolites extracted from various culture supernatants within 2 hours incubation, could be shown to be mutagenic in the Ames assay using the nitroreductase-deficient mutant strains TA98NR and TA100NR. In order to eliminate inefficient and lengthy solvent recovery steps, cell-free extracts from gut bacteria are being evaluated as alternatives which can be utilised directly in the plate incorporation assay together with preincubation in an anaerobic work station. The results obtained thus far will be presented and discussed.

SOME GENETICAL PROBLEMS IN THE LEPIDOPTERA

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This exhibit will be set out in the Nuffield Unit greenhouses, some of the insects being living and some pinned specimens.

Topics

(1) A possible explanation for "throwbacks", *i.e.*, unlinked modifiers in an unaccustomed background, occurring in a natural hybrid between *Papilio dardanus* and *Papilio phorcas* from Kenya.

(2) Speciation in the *Papilio machaon* complex. Taxonomists are quite definite that, for example, *Papilio machaon* and *Papilio hospiton* (Corsica and Sardinia) are good species, but naturally occurring hybrids are found and F2s can be produced in the laboratory.

(3) All-female broods in *Hypolimnas bolina* and *Acraea encedon*; possible causes.

(4) Industrial melanism and *Biston betularia*. The results of trapping the moth in one locality over 25 years. *F. carbonaria* has been reduced from 93·3 per cent to 64·5 per cent, but difficulties arise in relating this to soot and SO_2 levels.

Plus: One human problem. Why a 9-fold increase in centenarians in the last 30 years?

MUTAGENICITY OF SEVEN POLYCYCLIC AROMATIC HYDROCARBON COMPOUNDS IN *DROSOPHILA*

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The mutagenic response of adult *Drosophila* to treatment with carcinogenic polycyclic aromatic hydrocarbons is generally weak or absent. This is in contrast to the clearly positive results of the sex-linked recessive lethal assay to nearly all of the other pro-carcinogens. A larval assay was used in order to investigate whether or not the limited metabolic capacity for

pro-carcinogens in adults was shared by earlier stages in the life cycle. The compounds tested included: 2-acetylaminofluorene, 3-aminotriazole, auramine, benzidene, benzo(a) pyrene, cyclophosphamide, and o-toluidine. Feeding the compounds to third instar larvae produced a significant increase in sex-linked recessive lethals recovered in all of the compounds studied. No broad effects were detected. The results suggest that larval stages as well as adult stages should be investigated before concluding that a compound is not mutagenic in the *Drosophila* assay.

MOLECULAR ANALYSIS OF THE GENE FAMILIES CODING FOR THE STORAGE PROTEINS OF *PISUM SATIVUM* L

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The two storage proteins, legumin and vicilin, comprise a high proportion of the protein synthesised only in the developing pea seed. These therefore provide a good model system for the study of the control of the developmentally regulated genes in plants. Sequence data from both cDNA clones and the protein itself have been presented previously (Croy *et al*, *Nature, 295*, 76–79, 1982; Lycett *et al.*, *Nucl. Acids Res.*, *11*, 2367–2380). Further DNA sequence data from both classes of cDNA clones and from genomic clones encoding legumin will be presented.

Two long cDNA clones contain repeated elements within a region coding for an unusually polar region of the protein sequence. A previously reported clone has an apparent deletion of this region. Two separate genomic clones have been found to contain these repeats. This will be considered in terms of the type of inter and intra-specific variation that has been found in the vicilin gene family (Lycett *et al.*, *Nucl. Acids Res.*, *11*, 2367–2380).

The legumin gene contains three introns and the expected "CAT" and "TATA" boxes. There are also multiple polyadenylation signals—a common feature of plant genes (Lycett *et al.*, *FEBS Lett.*, *153*, 43–46, 1983) and a sequence like the "AGGA" box that has also been found in several other plant genes (Messing *et al.*, in *Genetic Engineering of Plants* (ed.) Hollaender, A., Plenum NY, 1983). These features will be considered with reference to those of other genes.

REPEATED SEQUENCE EVOLUTION IN *ALLIUM* GENOMES

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Much of the large-scale variation in DNA amount that is seen between species of the genus *Allium* can be explained in terms of repeated DNA sequences. We have observed changes in the organisation and amount of specific interspersed repeated sequences between different species and between varieties within a single species. Satellite DNAs have also been identified in a wide range of *Allium* genomes, and have been shown to possess an unexpected degree of sequence heterogeneity.

A STUDY OF THE *IN VIVO* PROCESSING OF THE CYANOGENIC β-GLUCOSIDASE (LINAMARASE) CONTROLLED BY THE *Li* LOCUS IN WHITE CLOVER

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Previous studies have shown that the locus *Li* controls the presence of linamarase protein in white clover plants (Hughes and Dunn, *Plant Mol. Biol.*, *1*, 169–181, 1982). In genetically competent leaf tissue, linamarase is the major high mannosyl glycoprotein which binds to

Concanavalin A. Antibodies were raised to enzymically active linamarase protein, SDS denatured linamarase protein and an α-chymotrypic digest of linamarase. These antibodies were used in an immunochemical study of the *in vivo* processing of linamarase. Linamarase glycosylation is inhibited by tunicamycin, an antibiotic which blocks the formation of N-glycosidic bonds.

RADIATION-INDUCED NON-DISJUNCTION IN FEMALE MICE

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Considerable efforts have been made to characterise the effects of environmental agents, such as X-rays, on meiotic chromosome non-disjunction in laboratory mammals. In the case of X-rays, although many investigations on radiation-induced non-disjunction have been carried out, much yet remains to be determined particularly with regard to its effects on female meiosis. It is known that non-disjunction can be induced in a dose-related fashion in immediately preovulatory oocytes of the mouse by small doses (10 to 50 cGy) of X-rays. However, such doses prove to be ineffective in earlier oocyte maturation stages. The work discussed in this paper was initiated to discover whether larger doses (100 to 600 cGy) of X-rays could induce non-disjunction in mouse oocytes at different developmental stages at the time of exposure.

The frequency of non-disjunction, as measured by the incidence of hyperploidy in one-cell embryos recovered from the female mice, rose significantly following exposure to these larger doses of X-rays. This effect was present in all maturation stages examined although the most mature stage proved to be the least responsive in terms of induced non-disjunction. Overall, a dose-related response was present but this was not so clear-cut for each maturation stage individually.

TESTING TRISOMIGENS IN *SORDARIA BREVICOLLIS* —SOME CONSIDERATIONS RELEVANT TO THE GENETIC RISK POSED TO MAN

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It has been suggested that the term "trisomigen" be used for substances which induce aneuploidy. A typical result of exposure to a trisomigen is a small increase in aneuploidy. Many substances have been identified which induce aneuploidy, but only a few produce large increases, *e.g.*, p-fluorophenyl-alanine in fungi; colchicine in hamsters. The characteristic small increases raises several questions of which two seem particularly important. Firstly, "How much of a hazard does exposure to a typical trisomigen pose to Man?" Secondly, "Can small increases be detected reliably and cheaply using available testing methods?"

We have been tackling these questions by studying the induction of aneuploidy following exposure of meiosis to dimethylsulphoxide (DMSO) both in normal strains and in strains predisposed to nondisjunction. The results of acute exposure and of protracted low level exposure to DMSO will be presented and the above questions considered in the light of our results.

THE CLASTOGENICITY OF CHROMIUM AND SELENIUM COMPOUNDS TO RAT TISSUES

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A range of reactive organic compounds have been proven to be clastogenic to rat lymphocytes exposed both *in vivo* and *in vitro*. We are now extending this technique to cover

inorganic compounds especially those derived from heavy metals such as the chromium and selenium compounds $K_2Cr_2O_7$ and Na_2SeO_3.

Rat lymphocytes have been scored for chromosomal aberrations after exposure to these compounds *in vivo* and *in vitro*. Bone marrow metaphases have also been scored from rats treated *in vivo*. *In vivo* treatment with $K_2Cr_2O_7$ was by three methods: intraperitoneal injection, intravenous injection into the tail vein, gavage. The maximum possible dose was found to vary according to the route used. Administration of Na_2SeO_3 was by intravenous injection only.

Both compounds were found to be clastogenic to lymphocytes exposed *in vitro* and bone marrow exposed *in vivo* with the exception of bone marrow exposed to $K_2Cr_2O_7$ by gavage. No significant increase in chromosomal aberrations was found in lymphocytes treated with the compounds *in vivo* by any route.

The implications of these results will be discussed.

(An MRC Project Grant and support from the Anglo Chemical and Ore company is acknowledged)

ROLE OF EXONUCLEASE III DURING *IN VIVO* RECYCLISATION AND DELETION OF LINEARISED PLASMID DNA MOLECULES IN *E. COLI*

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Plasmid linearised at a unique site by restriction endonuclease cleavage transforms *E. coli* with 10^2 to 10^3 times lower efficiency than corresponding covalently closed circular DNA. The majority of transformants obtained with linear molecules contain plasmids that are perfectly recyclised and retain the restriction site used for linearisation. However, a small proportion contain plasmids bearing deletions that extend various distances on either side of that site (R. Thompson and M. Achtman, *Mol. Gen. Genet.*, *169*, 49–57, 1979). Recyclization of linear pBR322 molecules *in vivo* following transformation into *E. coli* is primarily a recombination-dependent event (E. C. Conley and J. R. Saunders, *Mol. Gen. Genet.*, 1984, in press).

Deletants of pBR322 obtained in rec^+ or rec^- backgrounds fell into two classes, Type I which were less than monomeric length and which had lost the restriction site used for linearisation, and Type II which were greater than monomeric but less than dimeric length and which contained duplications and deletions of the pBR322 sequence. In wild-type *E. coli* the proportion of transformants obtained was approximately 95 per cent perfectly recyclised, 5 per cent Type I deletants and <0·05 per cent Type II deletants. However, transformation frequencies with linear pBR322 DNA were drastically reduced in an exonuclease III-deficient (*xth*1) mutant. Furthermore less than 2 per cent of the transformants contained perfectly recircularised plasmid molecules whilst the remaining 98 per cent contained Type II deletant plasmids of identical structure. This suggests that exonuclease III plays an important role in recyclising linear plasmid molecules and in the formation of monomeric (Type I) deletants.

REPAIR OF ALKYLATION DAMAGE IN DNA IN THE FUNGUS *ASPERGILLUS NIDULANS*

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The ability to repair DNA damage caused by simple alkylating agents is common to many organisms, from *Escherichia coli* to man. We are attempting to characterise these processes in the lower eukaryote *Aspergillus nidulans*, using both biochemical and genetic approaches. A system has been demonstrated which appears analogous to the adaptive response in bacterial cells, in which exposure of the fungus to low, non-toxic levels of mutagens, substantially reduces the mutagenic effects of a subsequent challenge dose. Biochemical analysis of cell free enzyme preparations has demonstrated the existence of a glycosylase specific for 3-methyladenine in DNA, which appears to be expressed constitutively and is not subject to product inhibition by 3-methyladenine. Attempts to further characterise these repair processes will be described.

MUTAGENIC REPAIR OF 8-METHOXYPSORALEN CROSSLINKS IN UV-EXCISION-DEFECTIVE *ESCHERICHIA COLI*

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Evidence was previously presented for a new pathway for the repair of 8-methoxypsoralen DNA crosslinks. The pathway, which is independent of the *uvrA* gene but deficient in *rep* mutants, has now been further characterised and shown to be more active in minimal than in nutrient growth media and to inhibited by acriflavine. Although crosslink repair is much reduced in *recA* bacteria, some still occurs as judged by the effect of acriflavine. By the same criterion, crosslink repair occurs in bacteria with point mutations in the *uvrA* and *uvrB* genes, in bacteria with a deletion covering the *uvrB* gene, and in *polA uvrA* bacteria. Bacteria with insertions rather than point mutations in the *uvrA* gene, although showing evidence of repair, demonstrated minimal inhibition with acriflavine suggesting the possibility that the *uvrA* gene product, even if enzymatically inactive, might be able to interact with DNA lesions in the presence of acriflavine and prevent crosslink repair. Crosslink repair in *E. coli* WP2 *uvrA* is associated with base pair substitution mutagenesis and may be characterised as an error-prone process. The *umuC* gene is not, however, required for crosslink repair in *uvrA* bacteria.

METABOLISM OF 4-CYANO-N,N-DIMETHYL-[^{14}C]-ANILINE (CDA) BY RATS AND MICE

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The metabolism of ^{14}C-CDA by rats and mice after single oral doses (18 mg/kg) has been investigated. The compound is rapidly absorbed and metabolised by both species. Rats and mice excrete 86 and 78 per cent respectively of the dose in the urine in 0–24 h.

^{14}C-CDA is extensively metabolised by the rat. Ten metabolities were separated from the urine by thin layer chromatography. None of these were unchanged CDA itself. The major metabolic processes were N-demethylation, N-acetylation, and *ortho*-hydroxylation followed by sulphation.

Other significant metabolites derive from further metabolism of N-methyl-4-cyanoaniline and N-acetyl-4-cyanoaniline at their methyl groups. In both cases, reactive intermediates are produced which react with glutathione. The consequent N-acetylcysteine conjugates have been isolated in the urine. In the case of the mercapturic acid conjugate of N-acetyl-4-cyanoaniline, there is evidence of even further metabolism involving C-S lysate. These processes have, in all, led to the formation of four novel metabolites.

The profile of metabolites in the mouse urine is much simpler; essentially only four metabolites are present. They are all metabolites which are produced by the rat. The reason for the simpler profile seems to be that the mouse does not N-acetylate 4-cyanoaniline and thus does not form the presursor for three of the metabolites.

The metabolism of CDA is discussed in relation to that of its structural analogue butter yellow (N,N-dimethylamino-axobenzene).

GENOTOXIC EFFECTS ON REPRODUCTION IN MALE RATS AND MICE AFTER SINGLE ORAL DOSES OF ETHYLENE GLYCOL MONOMETHYL ETHER (EGME)

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A widely used and important class of industrial solvents is the glycol ethers. A review by Hardin (*Toxicol., 27*, 91–102, 1983) reports studies demonstrating that some are teratogenic and others can case testicular atrophy or infertility. EGME is known to cause these effects with some indication of increased dominant lethality in rats and sperm morphology changes in mice after exposure of animals by inhalation (McGregor *et al., Toxicol. Appl. Pharmacol.*,

70, 303–316, 1983). The present study was initiated to investigate such changes in rats and mice in more detail after single oral doses of 500–1500 mg/kg body weight since, histologically, it has been shown that rats given a single oral dose of 500 mg/kg bodyweight produce spermatocyte changes within 24 hours of treatment (Creasy *et al.*, *Exp. Mol. Path.*, in press). Severe spermatocyte damage of the rat testes has been confirmed histologically in the present study. A reduction in sperm counts and increases in abnormal sperm have been found in both rats and mice. Fertility and pre-implantation losses appear to be affected in the rat but not in the mouse at these doses. Cyclophosphamide, the positive control compound showed the anticipated responses for sperm counts, sperm morphology changes, dominant lethality and male-mediated teratogenicity. The data suggests that rats and mice may be differentially sensitive to the effects of EGME and cyclophosphamide.

We thank MAFF for financial support.

INCREASED SISTER-CHROMATID EXCHANGE FREQUENCY IN PATIENTS RECEIVING SULPHASALAZZINE THERAPY FOR ULCERATIVE COLITIS

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Ulcerative colitis (UC) is a chronic inflammatory disease of the colon and rectum which may have its onset at any age. Its aetiology is largely unknown. Oral sulphasalazine (SASP, 2–4 g day^{-1}) is used as a maintenance therapy to prevent relapse. At present there is no satisfactory alternative to SASP in maintenance therapy and so on present practice individuals may take the drug for many years including their reproductive years.

We have found that UC patients who have been treated with oral sulphasalazine have a significantly raised frequency of sister-chromatid exchanges (SCE) in their circulating lymphocytes compared with matched controls. The micronucleaus (MN) frequency was unaltered.

The experimental design of our preliminary study does not distinguish between the disease and the treatment as a cause for the raised SCE level. We aim to resolve this problem by studying UC patients before therapy and at time intervals after therapy has commenced. We are also investigating the effect of SASP and its various metabolites on peripheral blood lymphocytes in *in vitro* cultures with and without metabolic activation. The results of these studies will be reported and discussed.

CHANGES IN CHROMATIN STRUCTURE DURING RETRACTION IN LAMPBRUSH CHROMOSOME LOOPS

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Lampbrush chromosomes are indigenous to amphibian oocytes, and their lateral loops are the sites of transcription. Towards the end of oogenesis, prior to maturation of the oocyte, these loops progressively retract into the chromosome axis. We are interested in two questions with regard to loop retraction: (a) what structural changes occur in the chromatin of the retracting loops? and (2) what changes in transcriptional activity accompany retraction?

Lampbrush chromosomes from maturing oocytes of *Triturus cristatus carnifex* were prepared for electron microscopy under 2 different ionic conditions. High salt conditions ($\simeq$70 mM monovalent cations) preserve higher order chromatin structure and chromosomes prepared under these conditions showed the highly condensed loops typical of late stage lampbrush chromosomes. Low salt conditions ($<$1 mM monovalent cations) cause destabilisation of higher order chromatin. Chromosomes prepared in low salt showed an increase in loop length of four-fold compared to loop lengths in chromosomes prepared under high salt conditions. This indicates that packaging occurs along the loop. However, electron microscopy also showed that the retracting loops had reduced, but still substantial levels of transcript associated with them. This unexpected finding, that retracting loops show packaging while still maintaining transcriptional activity, suggests that the packaging is in a more relaxed state than the solenoidal form characteristic of transcriptionally inactive chromatin.

IN VITRO DNA PACKAGING AND RECOMBINATION IN PHAGE T1

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Phage T1 is a representative of that large group of phages (including the T-even phages, P22 and P1) whose mature chromosomes exist as a collection of circularly-permuted DNA sequences. DNA metabolism in this group occurs by the formation of concatemeric intermediates from which "headfuls" of DNA are processively excised for packaging into heads. Formation of DNA concatemers requires phage-specified recombination functions.

A system, based on the complementation of concentrated T1 head-defective cell extracts, has been developed which allows the process of genetic recombination and DNA packaging to be reconstructed *in vitro*. This system will package endogenous concatemeric DNA present in the infected cell extracts to form infectious phage particles. Exogenously supplied T1 DNA, either as concatemer molecules or as virion DNA extracted from phage particles, is also packaged. Virion DNA is packaged by extracts of cells infected with T1 mutants totally defective in phage DNA synthesis and therefore virtually devoid of endogenous T1 DNA. However, this reaction only occurs with extracts containing active products from the two T1 recombination genes (genes 3·5 and 4). Since these two gene products are required for concatemer formation this result implies that virion DNA cannot be packaged *per se* but must first be converted to concatemer molecules. Physical studies have detected concatemers following incubation of T1 virion DNA with recombination-proficient extracts.

MERCURY-RESISTANCE PLASMIDS FROM BACTERIA ISOLATED FROM POLLUTED RIVER WATER

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Bacterial isolates from the River Mersey selected for tolerance to elevated concentrations of heavy metal ions (Hg, Pb, Cu, Cr, Zn, Ag, Cd, Co, Ni) have been tested for plasmid-specific resistance by conjugal transfer to *E. coli*. From the limited sample size (about 100 isolated strains) approximately 50 per cent were resistant to mercury (Hg) and only Hg-resistance was transferable by conjugation. Of the Hg-resistant strains 26 per cent (13 strains) were able to transfer Hg-resistance to *E. coli*.

Although isolated from a wide range of bacterial genera several of the Hg^R plasmids are closely-related on the basis of their restriction fragment pattern, while others are unique. These plasmids have been analysed in terms of their incompatibility groups, resistance to antibiotics and other heavy metals, phage inhibition and transfer characteristics. Several of the Hg^R determinants have been cloned into multi-copy plasmid vectors and their organisation is being investigated.

ANALYSING OF PHAGE T1 DNA METABOLISM IN AN *IN VITRO* DNA PACKAGING SYSTEM

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Phage T1 DNA is packaged *in vivo* from a concatemeric substrate by the "headful" mechanism which produces mature chromosomes with DNA sequences arranged in 3 cycles of circular permutations. The current model of T1 head assembly invokes enzymatic cleavage of endogenous concatemeric molecules initiated at a specific site referred to as *pac*. We have attempted to show that purified exogenous T1 virion DNA is cleaved at or near *pac* when incubated with packaging extracts derived from page-infected cells. These cleavages can be assayed by changes to the restriction fragment profile after agarose gel electrophoresis. The results suggest that *pac* site cutting, at least *in vitro*, is a relatively slow reaction. After incubation with infected-cell extracts T1 virion DNA is predominantly concatemeric and events leading to concatemer formation may obscure the effects of *pac* cleavage. When virion DNA is incubated with extracts from cells infected with a mutant unable to form concatemers a cleavage

occurs near the right end of the genome. This is probably unrelated to *pac*-initiated cleavage but may be analogous to site-specific cleavages reported for phages T4 and T7 under similar genetic circumstances.

PROPERTIES OF THE *UVR* PROTEIN OF *E. COLI*

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The *uvrA*, *uvrB* and *uvrC* genes of *E. coli* are involved in the process of excision repair, whereby damaged DNA is excised from the genome and the correct sequence restored. Acting together, the products of these genes make up a novel enzyme, termed an *exinuclease*, which carries out the early steps of the repair process. Cross-linking studies have been employed in an attempt to determine the roles played by the individual proteins of the complex, and the results of these studies will be described.

CHARACTERISATION OF THE UV PROTECTION AND MUTATION GENES OF THE I1 GROUP PLASMID TP110

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We have previously shown that TP110 can complement mutations in the *umuC/D* genes of *E. coli*, which result in slight UV sensitivity and block UV induced mutation. Use of Mu d (*lac amp*) to generate gene fusions has shown that the genes responsible (designated *imp*) are inducible by DNA damage and form part of the *rec/lex* controlled SOS response.

We have cloned the *imp* genes into suitable low copy number vectors, and, by sub-cloning and transposon inactivation, have located the genes within a 3·3 Md *EcoRI* restriction fragment. Properties of clones carrying these genes will be discussed, and the results of attempts to identify the gene products will be described.

RESIDENT-ENHANCED REPAIR OF BACTERIAL PLASMID DNA

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The survival of UV-irradiated plasmid DNA is dependent on the repair enzymes of the host cells into which it is transformed. However, survival can be increased about 10-fold for certain plasmids within the same incompatibility group if a damaged plasmid is transformed into a cell which already contains an incompatible resident. This effect is dependent upon host *uvrA* and *uvrB* genes, but not upon *recA*. By testing various combinations of plasmid pairs within the NTP16 incompatibility group and constructing deletion mutants, two areas of the plasmid have been implicated in the enhanced repair mechanism. One area lies close to the origin of replication and appears to be involved in the control of copy number, while the second area also lies close to the origin, on the opposite side to the first. By cloning these two areas separately into plasmids compatible with NTP16 we are attempting to confirm their role in the enhanced repair process, and perhaps identify the gene products involved. A model for resident enhanced repair will be discussed.

MUTAGENICITY OF CATECHOLAMINES AND RELATED CHEMICALS IN THE MOUSE LYMPHOMA L5178Y CELL tk^+tk^- MUTATION SYSTEM

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Dopamine [3,4-dihydroxyphenylamine] and epinephrine [3,4-dihydroxy-α-(methylaminoethyl)benzyl alcohol] are endogenous catecholamines with neurotransmitter functions.

Epinephrine, in addition, has humoral functions. These molecular structures are the basis for a large number of pharmaceutical agents. For this reason, dopamine and epinephrine were tested for mutagenic activity in the mouselymphoma L5178Y cell tk^+ tk^- mutation system described by Clive and Spector (*Mutation Res.*, *31*, 17–29, 1975). Using a 3 h exposure period (without provision of a supplementary activation system) and 3 day expression period, both compounds induced increases in the numbers and frequencies of colonies growing in the presence of trifluorothymidine (3 $\mu g \cdot ml^{-1}$). Dopamine showed the greater mutagenic potential, with a least effective concentration of about 15 $\mu g \cdot ml^{-1}$ (dopamine . HCl). This result qualitatively supports a very recent report (Moldeus *et al.*, *Mutation Res.*, *124*, 9–24, 1983) of mutagenic activity in lymphoma cells and DNA strand breaking potential in human fibroblasts. In view of the importance of these results with catecholamines, an investigation has been made of the structure-activity relationship of these and related chemicals. Particular attention has been paid to the possible involvement of *in vitro* artefacts, such as biochemically dislocated singlet oxygen ($^1O_2$) and hydroxy radicals ($OH^{\cdot}$).

SPECIFICITY OF ULTRAVIOLET MUTAGENESIS IN LAMBDA PHAGE

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We study the specificity of various mutagenic agents acting on the cI (repressor) gene of lambda phage. Mutagenesis of (1) the prophage in a lysogen measures mutagenic process in the *E. coli* host cell genome; (2) the free phage, allows the gene being mutated (on the page) and the host cell to be treated separately; (3) isolated phage DNA, allows treatment with enzymes, etc, before the DNA is repackaged and used to infect host cells. Mutants are identified by their clear plaque phenotype, mapped by phage crosses, and the DNA restriction fragments carrying the mutations sequenced to determine the changes in base order.

In targeted mutagenesis by ultraviolet light, the mutations are caused by lesions in the gene being mutated. The mutations are 2/3 transitions, and 1/6 each of transversions, frameshifts, and double events with either two nearby base changes or a base change and a nearby frameshift. Phage mutagenised by 313 nm light in the presence of acetophenone, a process said to produce mostly cyclobutyl pyrimidine dimers in the DNA, show mostly transversion and only a few transitions. Phage DNA irradiated with 254 nm light and photoreactivated to remove dimers before assaying, shows mostly transitions. Thus transitions, the major component of targeted ultraviolet mutagenesis, are not caused by the major lesion, the cyclobutyl dimer; we believe ultraviolet light induces transitions by a less common photolesion, the Pyr(6-4)Pyo photoproduct.

In nontargeted mutagenesis by ultraviolet light, assay of untreated phage in treated host cells leads to mutations. For host cells treated just enough to induce the SOS system, a ten-fold increase in mutation is mostly transversions, as shown by Jeffrey Miller and Brooks Low (personal communication) in the *E. coli lacI* gene. With more heavily irradiated cells, another ten-fold increase is caused by a second process which is independent of the *umuC* gene, and which induces mostly frameshift mutations.

ANALYSIS OF THE MODIFICATION AND MUTATION SPECTRA INDUCED BY THE CARCINOGEN N-ACETOXY-N-2-ACETYLAMINOFLUORENE IN DIFFERENT *E. COLI* STRAINS

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The covalent binding of an ultimate carcinogen to the DNA bases creates a premutational lesion which in most cases is processed in an error-free manner by the repair, replication and

recombination enzymes. At low frequency, however, a permutational lesion can be converted into a mutation.

We describe here the analysis of forward mutations induced in the tetracycline resitance gene of the plasmid pBR322 by directing the reaction of the carcinogen N-Acetoxy-N-2-acetylaminofluorene (*N*-Aco-AAF) to a small restriction fragment (*BamHI-SalI*) that is located in the proximal part of the antibiotic resistance gene (1). The mutation spectra obtained in different strains (wild type, *uvrA* and *umuC*) are compared.

The distribution of the AAF-adducts along the same *BamHI-SalI* restriction fragment was determined by using the $3' \rightarrow 5'$ exonuclease activity of T4 DNA polymerase. Indeed, we have shown that this activity is blocked in the vicinity of an −AAF adduct thus generating a pool of DNA fragments that are resolved and quantitated on sequencing gels (2).

From this work the following conclusions can be drawn:

(1) All G residues react with N-Aco-AAF (at frequencies that span more than one order of magnitude).

(2) Mainly frameshift mutations involving GC base pairs are obtained.

(3) The mutation spectrum is similar in wild type and *uvrA* cells, moreover, at equal level of modification the mutation frequencies are identical in both strains. In the *umuC* strain, the mutation frequency is about half of that observed in a wild type strain. Moreover, the mutation spectrum obtained in this strain differs significantly from the mutation spectrum obtained in a wild type strain.

(4) There is no direct correlation between the modification and the mutational spectra.

In the light of these results two pathways for frameshift mutagenesis will be discussed.

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MUTATIONAL ASPECTS OF HYBRID DYSGENESIS IN *DROSOPHILA*

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Hybrid dysgenesis occurs in the offspring of crosses between certain strains of *Drosophila melanogaster*. It is manifested as a set of abnormal traits including sterility, male recombination, mutation and chromosome rearrangement. These traits are seen in the offspring between two classes of Drosophila strains, M and P, but usually only when the P strain provides the male parent. A combination of genetic and molecular analyses have led to the identification of a transposable element, the P element, as the agent responsible for these effects. P strains have 30–50 P elements while M strains usually have none. Transposition is repressed in P strains but de-repressed in the germline of the offspring of the dysgenic cross.

Re-arrangements are often found in the chromosomes descendant from a dysgenic cross. Most chromosome rearrangements occur between sites where P elements already reside. The distribution of chromosome breakpoints consequently depends on the particular P strain used.

The frequency and distribution of mutations observed in a dysgenic cross depends on a number of factors. Some loci (*e.g.*, the *signed* locus) are always mutated at high rates. Molecular studies of P element insertions at the *white* locus show that there is sequence specificity for P element insertion. This may be the basis for some of the locus specificity observed. In some cases, particular loci of the P derived chromosomes are mutated at high rates. These mutations may be associated with chromosomal rearrangements and so reflect the strain-specific distribution of P elements within the chromosome (*e.g.*, *hdp* mutations are associated with rearrangement at the P element at 17C in the strain π_2). Finally, there is almost certainly some variation in the number and activity of P elements in different strains and this will affect the overall rate of mutation seen in dysgenic crosses.

MUTAGENESIS IN YEAST

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Our understanding of mutagenic processes in Saccharomyces lags well behind our understanding of these events in *E. coli*. Some 90 gene functions appear to be directly or indirectly involved in the control of yeast cell sensitivity to killing by mutagens. At the present time these functions can be grouped into those which are concerned with (a) various aspects of excision repair (b) the rejoining of DNA strand breaks and (c) mutagenesis. The relative importance of these in the restoration of viability depends on the inactivating agent. All mutagenic activity regardless of agent seems to be dependent on the third group of gene functions collectively called the "*rad6* group".

We now have a reasonably clear idea of the roles of excision and DNA replication in the initiation of UV- and chemical-induced mutagenesis but the details of the processes and the precise roles played in them by "*rad6* group" functions are far from clear. Cloning of some of these genes has already been achieved and the purpose of this presentation will be to discuss the progress being made towards an understanding of their functions in induced mutagenesis.

METHYLATION DNase I SENSITIVITY AND EXPRESSION OF RIBOSOMAL RNA GENE CLUSTERS IN HEXAPLOID WHEAT

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The genes coding for 18S and 25S ribosomal RNAs are highly reiterated in plants. In hexaploid wheat, the genes are localised at the nucleolus organisers (NOR) on chromosomes 1B, 6B, 1A and 5D. The number of genes at each NOR can vary substantially between plants. Many observations imply that the ribosomal RNA (rRNA) genes are in considerable excess over the minimum number required. Not all rRNA genes are equally methylated at cytosine residues in CCGG sequences. Some genes are methylated at all such sequences. One CCGG is preferentially non-methylated in a subset of rRNA genes. From studies using nucleolus size to estimate rRNA gene activity, it can be concluded that inactive rRNA genes are methylated at more CCGG sites and organised in chromatin more resistant to DNase I than active rRNA genes. Furthermore the state of methylation of a particular gene cluster is not invariant. It changes in genetic backgrounds varying in rRNA gene number. Dominance of one NOR over another is also associated with changes in rRNA gene methylation and DNase I sensitivity.

There is considerable length variation in wheat in the "spacer" regions between the repeating rRNA gene units due to different numbers of a 130 bp subrepeat. However, within an NOR there is much less spacer length variation than between NORs. This is evidence for the existence of active homogenising mechanisms within the arrays of repeating genes. Some wild tetraploid wheat populations from Israel contain rRNA genes with many different spacer lengths while other populations contain very few spacer length polymorphisms.

PROLAMIN GENES OF CEREALS; MULTIGENE FAMILIES WITH UNUSUAL MOLECULAR STRUCTURE

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About 40 to 50 per cent of the endosperm proteins of cereal grains are prolamin storage proteins. They are therefore one of the quantitatively most important group of proteins in our agriculture biosphere and they also influence the quality of cereal grain. We have classified the prolamins of the Triticeae into three major groups: the sulphur-rich, the sulphur-poor and the high molecular weight prolamins. We have mapped the loci encoding these proteins in barley and rye. We have further investigated the nature of these by isolating cDNA and genomic DNA clones related to each of the major sub-groups of proteins from either barley,

rye or wheat. Southern blotting has shown that each group is encoded by a multigene family. Sequencing of the DNA clones allied to direct sequencing of the prolamin polypeptides has revealed that each family is made up of genes that contain within them a number of repeated sequences. The relationships between the families and some speculations regarding the origin of the genes will be presented.

PEA SEED STORAGE PROTEINS AND THEIR ENCODING GENES

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Pea (*Pisum sativum* L.) seeds, besides their relevance to agriculture, contain differentiated tissue in which a relatively small number of gene products accumulate in large amounts during the development of the organ. They are thus of interest as model system for studying the developmental regulation of gene expression in plants.

The proteins found in large amount in pea seeds have primarily a storage role, and contain as major components legumin and proteins of the vicilin fraction (vicilin and convicilin). These proteins are synthesised on membrane-bound polyribosomes and are transported to protein bodies during the cell expansion phase of seed development. Besides removal of "leader" sequences, post-translational proteolytic modification involving cleavage at asparagine-X bonds occurs in legumin and in some vicilin polypeptides.

Genetic variation in polypeptides of legumin, vicilin and convicilin has allowed the encoding genes to be mapped onto the pea genome by classical techniques. The loci for these proteins are not contiguous, and thus the genes must be independently regulated. In agreement with this conclusion, studies during seed development indicate differing patterns of synthesis and accumulation for the different storage protein polypeptides, and differing levels of different mRNAs within an overall pattern of mRNA accumulation and decay as seed development proceeds.

Transcription of isolated nuclei from developing seeds and leaves has shown that gross regulation of seed protein gene expression occurs at the transcriptional level, but indicated that further control was exerted at the stage of post-transcriptional processing. In the later stage of seed development control at other stages (mRNA lifetime, translation) may also be important.

Several genes encoding legumin polypeptides have been cloned, and the sequence of one shows it to possess the typical features of a eukaryotic gene.

IS CAULIFLOWER MOSAIC VIRUS STILL A POTENTIAL VECTOR FOR GENES INTO PLANTS?

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Cauliflower mosaic virus (CaMV) is the type member of a group of plant viruses which contain double-stranded DNA. Two approaches have been used to study its potential as a vector for taking foreign genes into plants. The effect of site-directed deletions or insertions on the infectivity of CaMV DNA to plants shows that most regions of the viral genome are essential for full replication of the virus. However perturbations can be made in some parts of the viral genome and attempts have been made to insert genes. Secondly the molecular biology of CaMV has been studied extensively. The DNAs of several isolates have been sequenced and six potential coding regions have been recognised. Functions have been ascribed to three of these. Transcription of the DNA is asymmetric, one of the transcripts being the mRNA for one of the gene products; mRNAs have not been found for the other gene products. The other major transcripts is full length and has a terminal direct repeat of about 180 nucleotides. There is considerable evidence that this is the template for DNA synthesis using a reverse transcription mechanism similar to that of retroviruses. The implications of all these findings on the use of CaMV DNA as a gene vector will be discussed.

THE USE OF RECOMBINANT DNA TECHNOLOGY IN DNA REPAIR STUDIES IN *ESCHERICHIA COLI*: A REVIEW

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The review will illustrate how recombinant DNA technology has provided novel information on the mechanisms and regulation of DNA repair in *Escherichia coli*. In its initial stages these techniques were used to increase gene copy number and expression, thus easing the problem of protein purification for *in vitro* biochemical mechanistic studies. They were, however, soon employed in the construction and analysis of defined DNA substrates for reactions involving components of excision, recombination and mutagenic DNA repair. Defined changes in DNA sequences encoding DNA repair proteins were also engineered, both *in vitro* and *in vivo*, often with the aid of transposable elements. These combined approaches have contributed to studies of gene expression, gene organisation and protein activities. Underlying most of this work have been technical innovations which permit simple analyses of the composition and activities of heterogeneous mixtures of proteins, DNA or RNA. A molecular description of the *E. coli* SOS response will demonstrate the application of this technology.

TRANSFER OF RECOMBINANT DNA MOLECULES INTO MAMMALIAN CELLS FOR REPAIR AND MUTAGENESIS STUDIES

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Specific alterations in DNA sequence, structure and continuity may be achieved *in vitro*, particularly within small DNA species such as plasmids. A number of recombinant plasmids are available which contain genes coding for dominant, selectable functions in mammalian cells. Such plasmids may be introduced into many different types of cultured mammalian cells by DNA-mediated gene transfer techniques. The subsequent use of appropriate selective media ensures that only those cells maintaining and expressing the plasmid-encoded genes will survive.

The effect of *in vitro* damage to the DNA sequence of a given selectable gene may be assessed *in vivo* since, if the damaged sequence is not correctly repaired by cellular processes, the gene cannot function and the recipient cell will not survive in the selective medium. This approach may be used to determine the relative importance and mechanism of repair of lesions introduced by a wide variety of mutagens/carcinogens. As an illustration of this, evidence will be presented on the repair of double-strand DNA scissions in normal and radiosensitive (ataxia telangiectasia) human cells. In a similar way, defective plasmids carrying specific deletions/insertions in the selectable gene have been used for the analysis of cell-mediated recombination.

Conversely, the introduction of intact selectable genes into mammalian cells may be used to provide defined targets for subsequent *in vivo* mutagenesis. Different cell populations exposed to a recombinant plasmid will provide a collection of cell lines with the same gene integrated into different genomic locations—an exciting prospect for the analysis of mutational processes at the molecular level. Mutation data obtained with a hamster cell line containing a single plasmid-encoded gene will be presented.

SOMATIC AND GERM LINE MUTATIONS AT THE HPRT LOCUS IN RODENTS AND MAN

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Full-length cDNA clones of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene from man have been employed in studies of the normal and mutated gene. The normal human gene structure has been determined by identifying and characterising genomic clones

from lambda libraries using these cDNAs as probes. cDNA probes were used in conjunction with data from the normal gene to analyze the HPRT locus in Lesch-Nyhan patients. Southern blot analysis has detected two patients with deletions in the 3′ portion of the gene, one patient with a complete gene deletion, one with alterations in the second and/or third exons and one with alterations in exons four and five.

Knowledge of normal HPRT gene structure allowed construction of recombinant expression vectors for transfer of this gene into cultured cells. Minigenes which contain a viral LTR promoter, the eukaryotic metallothionein promoter or the natural HPRT promoter fused to full-length human cDNA have been constructed. Each minigene can transfer a functional HPRT gene into cultured cells. Transgenic mice have been produced using these minigenes. Expression of human HPRT in these mice is currently under investigation.

CELLULAR ONCOGENE ACTIVATION AND MULTISTAGE CARCINOGENESIS

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Some of the most significant advances ever achieved in research into the genetics of carcinogenesis have been made within the past four years. The central discovery on which all this recent progress was based was the finding that purified DNA from a proportion (~20 per cent) of solid human tumours contains activated endogenous genes of the *ras* family which, on transfection, can cause malignant transformation of a non-malignant mouse cell line (NIH 3T3). Activated *ras* genes discovered in human tumours, as well as those found in the corresponding acute rodent sarcoma viruses (HaSV and KiSV) are distinguishable from their normal counterparts solely by a single point mutation in either codon 12 or codon 61, resulting in an amino-acid substitution in the encoded p21 *ras* protein. The function of p21 *ras* in normal cells and the consequences of mutational activation are not yet understood. Nevertheless, it is now generally accepted that *ras* gene activation plays a significant role in the genesis of at least some carcinomas and sarcomas.

Carcinogenesis is almost invariably a multistage process in both humans and carcinogen-treated laboratory rodents. Consequently, because activated *ras* genes have so far been studied only in primary tumour tissue or derived cell lines (*i.e.*, at the endpoint of carcinogenesis) it is not yet clear precisely at what stage in the process activation occurs and therefore to what extent these genes are responsible for malignant transformation. Moreover, since about 80 per cent of solid human tumours do not appear to contain activated oncogenes detectable in the NIH 3T3 system, there are as yet still no clues as to the genetic basis of the majority of malignancies.

In order to provide an approach to answering some of the above questions, the efforts of my group have been directed towards developing improved cell culture models for carcinogenesis which permit the delineation of the various stages in the process (see *Nature*, *299*, 633). Using these systems we have shown that activated *ras* genes are stage-specific with respect to their transforming properties (*Nature*, *304*, 648). These and more recent results, particularly those which throw further light on the genetic basis of malignant transformation by mutagenic chemicals, will be discussed.

MODULATION OF REPETITIVE DNA IN THE MAIZE GENOME

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The repetition frequencies of a number of cloned DNA sequences of maize have been shown to be polymorphic among inbred corn lines. The extent of this copy number variation and its stability in crosses have been determined. The ten clones used as probes represent a spectrum of the repetitive component of the maize DNA from less than 100 to more than 100,000 copies per genome. A comparison of the quantities of each sequence in various inbreds

showed:
(1) There was no correlation between copy number and copy number variability.
(2) There was no generalised control over repeat sequence copy number in different genotypes.
(3) The functionally related 5s and rDNA genes were not co-ordinately varied for copy number.
The copy number polymorphisms were stable in individuals of an inbred line. In crosses between inbred lines the clones that were invariant in copy number and some copy number variant sequences were present in the expected amounts in the F1 (average of parentals). However in some crosses the F1's deviate considerably from the expected values, with both increases and decreases in copy number being observed.

THE FLAX GENOME—ITS PLASTICITY AND RESPONSE TO STRESS

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A number of DNA sequences which vary between the environmentally induced genotrophs of the flax variety "Stormont Cirrus" have been isolated. These include the ribosomal DNA, 5s DNA, a part of a sequence which acts like a transposable element and a number of uncharacterised repetitive sequences. In addition to these, a number of cloned sequences which did not vary between genotrophs have also been isolated. Examples of highly repetitive, tandemly arranged sequences are included in both variable and constant classes. Even within a sequence set, namely the 5s DNA, particular recognisable subsets appear to be differentially affected.

The DNAs from normal seed derived plants, callus tissue and the progeny of regenerated plants have been compared using a number of probes characterised in the genotroph study. The same two classes appeared, that is, sequences which varied between genotrophs also varied between plant, callus and regenerants, while those which were constant in the genotrophs were also invariant between plant, callus and regenerants. The two classes are also apparent when a comparison was made between the DNAs from various flax and linseed varieties and the supposed progenitor of flax *Linum bienne.*

From these results it is proposed that the flax genome is compartmentalised into 2 parts, one of which is variable and the other which is constant. The effect of "stress" is to cause change in the variable component which is responsible for the phenotypic variation subsequently observed.

DNA BINDING OF CYCLOPHOSPHAMIDE PROCEEDS THROUGH PHOSPHORAMIDE MUSTARD

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Cyclophosphamide (CP) is a bifunctional alkylating agent widely used in cancer chemotherapy. The compound is extensively metabolised in the body; up to nine different metabolites have been identified to date. The active form of CP is thought to result from mono-oxygenase attack of the parent compound to yield 4-hydroxy-CP. This metabolite is spontaneously converted to adolphosphamide and then phosphoramide mustard (PM) and acrolein. PM is thought to be partly responsible for the cytotoxic action of this drug. We have found that *in vitro* [^{3}H]CP covalently binds to DNA in the presence of a rat liver "S9" metabolising system. Acid hydrolysis of this DNA yields a base adduct which is not retained on a reversed-phase HPLC column. If this acid hydrolysed DNA is subsequently treated with acid and alkaline phosphatases then the adduct is retained on the column indicating that the phosphatases may have removed the phosphate group from the adduct. Reaction of PM with deoxyguanosine followed by phosphatase treatment also yields a single adduct with an identical retention time. This suggests the binding of CP to DNA proceeds via PM *in vitro.* Preliminary studies in rats administered [^{3}H]CP indicates that the major liver DNA adduct is the same as that generated *in vitro* with PM.

N-ACETYLATION AND MUTAGENICITY OF BENZIDINES IN THE AMES ASSAY

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Benzidine (BZ), a human bladder carcinogen and a rodent hepatocarcinogen, is mutagenic in the Ames *Salmonella*/microsome assay. In rodent liver BZ is metabolised to *N*-acetylbenzidine (ABZ) prior to further metabolism and DNA binding. However, the S9 mix used in the Ames assay shows no acetylating activity, thus the metabolism of BZ to a mutagen in the Ames assay may differ from the *in vivo* carcinogenic activation of BZ in rodent liver.

BZ and two of its 3,3 -distributed analogues *o*-tolidine (OT) and *o*-dianisidine (OD) were tested in the Ames assay with and without added acetyl coenzyme A (acetyl-CoA). The addition of acetyl-CoA was observed to increase the mutagenicity of BZ four to five-fold, this may be a result of the generation of ABZ which is more mutagenic than BZ. In the presence of acetyl-CoA, OT showed a smaller increase in mutagenicity than BZ. However, OT acetylation was only 37 per cent of that observed with BZ. OD mutagenicity was unaffected by acetyl-CoA. OD was acetylated least by the metabolising system (16 per cent of BZ acetylation) and acetylated OD species showed lower mutagenicity than OD itself.

These results indicate that the potential mutagenicity of BZ which is activated via acetylation may be under-estimated in the usual Ames assay protocol. The inclusion of acetyl-CoA or testing *N*-acetyl derivatives of aromatic amines may provide a better indication of their likely *in vivo* genotoxicity. In comparing the mutagenicity of BZ with OT and OD, it appears that *N*-acetylation may be less important for Ot and OD than for BZ. If the same effect applies to whole animal metabolism, the liver DNA bound species of OD and OT may have a significant contribution from a non-acetylated adduct, or the DNA binding of these two species may be much reduced compared with BZ.

MUTAGENICITY OF INDOOR AIR-CONTRIBUTION FROM TOBACCO SMOKING AND FROM WOOD BURNING

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Samples of airborne particles were collected in a well-ventilated living room when the room was heated by electricity and when heating was achieved by wood burning. For both heating conditions samples were collected in the presence and absence of tobacco smoking.

Extracts of the samples were tested for mutagenicity in the Ames' *Salmonella* assay. The results showed that the particles collected when moderate smoking occurred were several times more mutagenic than samples from traffic polluted urban air. Wood heating in open fire places did also produce particles of increased mutagenic activity, but this effect was moderate as compared to the effect of tobacco smoking.

A NEW AGROBACTERIUM VECTOR FOR PLANT TRANSFORMATION

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The large plasmids of *Agrobacterium tumefaciens* confer on that bacterium the ability to incite tumours on a wide variety of plants. The molecular basis for the transformation is the insertion and expression of a large segment of Ti plasmid DNA in the host nuclear genome. Genes responsible for causing tumourous growth have been identified, and can be deleted without affecting the ability of Ti plasmid sequences to be transferred to the plant cell. Two types of transforming vector have been constructed that contain a chimaeric kanamycin resistance gene for selecting transformants, deleted oncogenes that allow for the regeneration

of morphologically normal transformed plants, restriction sites for inserting passenger DNA, and a strong plant viral promoter for the expression of passenger DNA sequences. These vectors are easily used and transform plant protoplasts ar a frequency of approximately 1–10 per cent.

THE USE OF AN AUTOMATED BACTERIAL MUTAGENICITY ASSAY IN THE DETECTION OF CHEMICAL MUTAGENS

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Mutascreen is a new automated mutagenicity assay for the detection of mutagenic activity in chemical, environmental as well as in biological samples.

The Mutascreen system consists of an automated analyzer and sample dispensing unit, a desk-top computer and printer together with a complete computer software. The analyzer's dispenser dispenses samples, all the necessary reagents, bacteria and S-9 mix into the reaction cuvettes. The bacterial growth is monitored by a vertical pathway turbidimeter. Bacterial growth is transformed and shown in graphical form by the desk-top computer. The growth curves are used to indicate samples' mutagenicity, toxicity and the presence of the possible growth factors in the sample.

The estimation of the mutagenic activity of a sample is made by the computer based on the generation times of the auxotrophic and the prototrophic populations, as well as on the time of appearance of the prototrophic growth and on the size of the bacterial population at that time.

The assay system can carry out up to 200 analyses within 24 hours and allows the user to select freely the concentrations of samples, the number of bacterial strains as well as the amounts of S-9 mix.

Numerous samples have been tested with the Mutascreen and the results show good correlation with the conventional methods.

THE USE OF COMPETITIVE SUBSTRATES TO PROBE THE BACTERIAL MUTAGENICITY OF BENZIDINE AND DIAMINOTERPHENYL

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The structurally related compounds benzidine (BZD) and 4,4'-diaminoterphenyl (DAT) were evaluated for mutagenic activity using the Salmonella/Plate Assay, together with strain TA1538 and uninduced liver S9 fractions prepared from rats and mice.

BZD was only active in the presence of S9-mix, and mouse derived preparations were the most effective in this respect. In contrast DAT was found to be a potent direct acting reactive intercalator. Low concentrations of uninduced S9-fraction (0·3–2·5 per cent v/v) effectively reduced this mutagenic activity. This was an enzymic process which was inactivated by heating the S-9 fraction.

However, the use of higher concentrations of S9-fraction (2·5–30 per cent v/v) appeared to result in the generation of an additional mutagenic species. Unlike BDZ, rat liver S9 was more effective than mouse S9 in this step.

The competitive substrates, aniline, dexamphetamine and the thiol-scavenger p-chloromercurobenzoate were used to selectively block C-hydroxylation, N-hydroxylation and N-acetylation respectively. The results obtained indicate that N-acetylation is critical for the *in vitro* formation of the major mutagenic metabolites of BZD. However, N-acetylation does not appear to play an essential role in the reactivation of DAT. Instead C-hydroxylation seems to be a critical step.

Further evidence to substantiate this conclusion was obtained through the use of dog S9 which, although devoid of N-acetyl-transferase activity, effectively reactivated DAT to a bacterial mutagen *in vitro*.

SCE TESTS OF BUTTER YELLOW, 4-CYANODIMETHYLANILINE, BENZIDINE AND DIAMINOTERPHENYL USING CHINESE HAMSTER V79 CELLS IN THE PRESENCE OF S9-MIX

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At sub-toxic concentrations DAB, and BZD induced statistically and biologically significant, dose-related increases in the frequency of SCE within V79 4K1 lung fibroblast cells, in the presence of S9-mix. The results were quantitatively very similar when S9-fractions were derived from the livers of male rats pretreated with aroclor 1254, or with a combination of phenobarbitone and βnaphthoflavone. Considerable inter-cell variation in the frequency of SCE was observed after treatment with BZD. This effect was reproducible and unique to this compound within the study. These results may have been due to the presence of subclones of varying sensitivity to the mutagenic metabolites of BZD in the original cell cultures. Alternatively the data may be indicative of an S-phase specific effect. It is possible that the metabolites of BZD affect DNA replication at the level of some components of the replicative process rather than affecting the DNA molecule itself.

The structurally related compounds CDA and DAT were inactive in the SCE assay. The use of larger S9 concentrations, or prolonged treatment periods were without effect. Furthermore, negative results were obtained irrespective of the nature of the inducing agent utilised for S9 preparation.

DAT was also inactive in the absence of S9-mix, although the compound has been found to be a potent direct acting mutagen in bacterial mutation tests.

MUTAGENICITY TESTING WITH SOMATIC CELLS OF *DROSOPHILA*

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In *Drosophila* larvae, mitotic recombination and mutation may be induced in the mitotically active precursor cells of adult structures. In individuals with a suitable genotype such events initiate clones which become recognisable as mutant spots on the adult body. We used trans-heterozygotes for the 3rd-chromosomal recessive wing cell markers *mwh* and *flr*. The spontaneous rate of clone initiation is about 5×10^{-5} per cell and per mitotic cycle as determined from clone size and clone frequency of *mwh* spots. Mutagen treatment increases the frequency of clones. By its sensitivity, the system lends itself as a rapid *in vivo* assay for mutagenicity testing. Some 80 chemicals have been tested so far by feeding and by gas exposure. The system detects a wide range of mutagens including monofunctional alkylating agents, cross-linking agents, mutagens forming large adducts, DNA breaking agents, intercalating agents, base analogs, antimetabolites, spindle poisons, etc. Many promutagens needing metabolic activation induce spots. Actual studies are focused on optimal tests procedures. We determined empirically that the analysis of wings from some 20–25 flies is sufficient to assess with statistical significance an induced tripling of the spontaneous clone initiation frequency.

BIOMETRICAL GENETIC ANALYSIS OF LEARNING IN *DROSOPHILA*

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Demonstrations of learning in *Drosophila melanogaster* raise questions about the adaptive value of behavioural modifiability in this species. One method of assessing the adaptive significance of a behavioural trait is the examination of its genetic architecture. The genetic architecture of olfactory discriminative avoidance conditioning has been examined in a series of inbred lines using the single test-cross design. The genetic architecture was characterised by low amounts of additive genetic variation, some epistatic variation and strong directional dominance for increased learning ability. This genetic architecture suggests that there may be a close association between evolutionary fitness and this learning phenotype. The implications of these results will be discussed.

PARAMUTATION IN *ANTIRRHINUM MAJUS*: AN INTERACTION BETWEEN TWO TRANSPOSABLE ELEMENT SYSTEMS?

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Paramutation has been studied at the *R* and *B* loci of *Zea mays*, the *sulfrea* locus of *Lysopersicon esculentum*, and the *nivea* locus of *Antirrhinum majus*. In the latter system crosses between the stable white nivea 44 and the highly variegated nivea 53 line revealed more than 99 per cent of the F1 progeny to be white. This was unexpected but the phenomenon was shown to be heritable (Harrison and Carpenter, 1973, *Heredity 31*, 309). The highly variegated *niv* 53 allele apparently had undergone a mutation under the influence of the stable *niv* 44 allele to become stable white.

In order to analyse this phenomenon molecularly, we cloned both alleles and determined their DNA sequence.

Niv 53 is due to the 17 kb long Tam1 element integrated 17 bp 5′ upstream of the TATA box of the *chalcone synthase* gene (*nivea* locus). Upon integration of Tam1 into the locus a 3 bp duplication is formed. In the *niv* 44 allele the 5·6 kb Tam2 element is integrated into an exon-intron boundary of the *chs* gene, also generating a 3 bp duplication. The F1 progeny of the cross has not yet been analysed molecularly. However, the similarity of the DNA sequences of the termini of Tam1 and Tam2 as well as some Southern blotting experiments suggest that paramutation in *A. majus* might be due to physiological interaction between the Tam1 and Tam2 systems, rather than to a mutational change of the *niv* 53 allele.

THE IMPORTANCE OF CONSIDERING INACTIVATION BY MEMBRANE DAMAGE AFTER NEAR-UV IRRADIATION OF *ESCHERICHIA COLI* WHEN ASSESSING MUTAGENESIS

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We have demonstrated previously that near-UV, but not far-UV, irradiated DNA repair-proficient *Escherichia coli* are inactivated by lower fluences if viability is assessed on defined media plates containing a high concentration of inorganic salts compared with viability assessed on similar plates containing a low concentration of inorganic salts or on complex media (S. H. Moss and K. C. Smith, 1981, *Photochem. Photobiol. 33*, 203–210). This has since been correlated with leakage from cells after inactivating fluences of near-UV, but not far-UV, radiation (L. R. Kelland, S. H. Moss and D. J. G. Davies, 1984, *Photochem. Photobiol. 39*, 329–335).

The present work investigates the possibility that the number of observable mutants obtained after broad-band near-UV irradiation of repair-proficient cells, by the "classical" method of reversion of prototrophy, might be reduced due to membrane damage being a significant factor under the conditions of the experiment. We report here on attempts to protect near-UV irradiated cells from membrane damage by firstly lowering the inorganic salt content of the minimal media plates used for the assessment of mutagenesis and viability, and secondly by the use of salts buffer solutions present in the plating media, of differing total molarities. Our studies indicate that the inorganic salt content of the minimal plating media plays an important role in the scoring of mutations, and preliminary data suggest that near-UV radiation-induced membrane damage may be important in the estimation of the induced mutation frequencies.

THE CHINESE HAMSTER GLUTAMINE SYNTHETASE GENE

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Glutamine synthetase, a key enzyme in the integration of nitrogen and energy metabolism, is inhibited by methionine sulphoximine. Multistep selection of Chinese hamster ovary cells

in methionine sulphoximine resulted in glutamine synthetase gene amplification, allowing us to clone genomic DNA sequences coding for the glutamine synthetase gene in the recombinant plasmid pGS-1 (Sanders and Wilson, *EMBO Journal*, *3*, 65–71).

Mutant cell line KG1MSC4-M contains 1000 copies of the glutamine synthetase gene, with an amplified unit of at least 50 kbp. Using cDNA probes we have determined the location of glutamine synthetase gene sequences within pGS-1 and the direction of transcription. Glutamine synthetase coding sequences also lie outside the CHO DNA cloned within pGS-1 and we are analysing these sequences using lambda recombinants. The GS gene appears to cover 9 kbp of the genome and is currently being sequenced. Northern blot analysis detects at least three abundant mRNA transcripts in mutant cells which hybridise to glutamine synthetase genomic DNA probes.

We are currently looking for karyotype abnormalities, which are often associated with gene amplification. We are also analysing the processes involved in gene amplification and the regulation of glutamine synthetase gene expression. The glutamine synthetase gene is differentially expressed in different tissues and responds positively to the presence of glucocorticoid hormones.

CHROMOSOME STUDIES IN RAT LIVER CELLS EXPOSED TO GENOTOXIC CHEMICALS *IN VIVO* OR *IN VITRO*

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The results of part of the UKEMS second collaborative study are reported.

The compounds were tested in a rat liver *in vivo/in vitro* assay and also in a rat liver (RL_4) *in vitro* assay for their ability to induce chromosome aberrations and sister chromatid exchanges (SCE).

Young male Wistar rats were given single i.p. doses of 7,12-dimethylbenzanthracene (DMBA), cyclophosphamide (CPM), 4-dimethylaminobenzene (DAB), 4-cyanodimethylaniline (CNA) or benzidine. Four hours after dosing, primary cultures of liver tissues from each animal were prepared on microscope slides and, after an appropriate period in culture, these were processed and stained. In the *in vitro* assay, the cytotoxicity of the test compounds to rat liver (RL_4) cells was established first and then cells on microscope slides were exposed to DAB, CDA, benzidine or 4,4-diaminoterphenyl (DAT) for 24 h before processing and staining them.

In the *in vivo/in vitro* assay, under the experimental conditions described, CPM and DMBA induced both chromatid aberrations and SCE in cells from animals exposed to these compounds. Benzidine and DAB induced a marginal dose-related increase in the percentage of cells showing chromatid and chromosome breaks and benzidine increased the frequency of SCE slightly. However, these changes were so small that it is concluded that neither benzidine, DAB or CDA significantly increased the frequencies of chromosome damage or SCE.

In the rat liver (RL_4) *in vitro* assay, benzidine induced a significant dose-related increase in the frequency of all types of chromosome damage and SCE, but DAB, CDA or DAT did not do so.