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TRANSFER OF FOREIGN GENES INTO THE MOUSE GERM-LINE

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We have produced several lines of mice carrying a foreign gene, the rabbit adult B-globin gene, in the germ-line. The gene was introduced by microinjecting a cloned rabbit DNA fragment into the pronuclei of fertilized mouse eggs, and allowing them to develop to term in foster mothers. Nine mice carrying the foreign gene in a somatic tissue were identified by Southern blot hybridization, and at least seven of these mice transmitted the gene to a fraction of their offspring. Transmission to a third generation has now been observed in one of the lines. Most of the mice contain multiple copies of the injected rabbit DNA clone (typically five to twenty copies), and these copies appear to be arranged in tandem arrays that are integrated into a mouse chromosome. The site of chromosomal integration has been identified by *in situ* hybridization for two of the original nine positive mice; in one case, integration has occurred in chromosome 1, and in the other case in chromosome 12. We are now investigating the expression of this foreign gene in erythroid and non-erythroid tissues of the mice.

THE STRUCTURE AND EXPRESSION OF THE GLUTAMINE SYNTHETASE GENE IN CHO CELLS

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Starting with the wild type CHO–K1 Chinese Hamster Ovary cells, variants have been selected with greatly increased levels of glutamine synthetase. The most extreme of these variants (1MS) has approximately 1000-fold increased levels of glutamine synthetase, the enzyme now comprising more than 30 per cent of soluble proteins. Digestion of DNA from K1 wild-type and 1MS variant cells with a variety of restriction nucleases shows that about 100 kilobase pairs of DNA have been amplified in the variant cells. These cells have been used to obtain data on the glutamine synthetase gene structure and function.

A STUDY OF MUTANT STRAINS OF *Sordaria brevicollis* EXHIBITING GREATLY INCREASED FREQUENCIES OF MEIOTIC ANEUPLOIDY

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A system for detecting meiotic aneuploidy has been developed in *Sordaria brevicollis* using complementing spore colour mutants. Using this system, strains have been isolated in which the aneuploid frequency is increased from a control level of 0·1 per cent to 30 per cent in one case and 10 per cent in another.

The mutations are dominant and do not appear to bring about the increase through a defect in chromosome pairing because recombination frequency is more or less normal.

AN EXTENSION OF THE ANEUPLOID TESTING SYSTEM IN *SORDARIA BREVICOLLIS*

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An aneuploid testing system has been described, in *Sordaria brevicollis*, which makes use of complementing spore colour mutants at the buff locus, on linkage group 2 (Bond, 1976, *Mut. Res. 37*, 213–220). It is now possible to examine aneuploid induction with a second linkage group in this organism. This is due to the existence of complementing spore colour mutants, at the *grey*-6 locus, on linkage group 4. There are five alleles and each one complements the other four, at least to some degree.

Work has been done to characterise spontaneous aneuploid frequencies in crosses of these mutants and to determine the effect of an aneuploid inducing agent, p-fluorophenylalanine (pFPA), on such crosses. These results are compared with those obtained using linkage group 2 markers. Attempts are being made to develop a random spore plating method using closely linked auxotrophic markers on linkage group 4.

REGULATION OF YOLK PROTEIN GENE EXPRESSION IN *DROSOPHILA*

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There are three major yolk polypeptides (YPs) accumulated in the *Drosophila* oocyte. They are synthesised in the ovary and fat body of the adult female, and each is coded by a single gene located on the X-chromosome. Males do not normally synthesise the YPs, but they can be induced to do so by the steroid hormone, 20-hydroxy-ecdysone. The expression of the YP genes in male, female and intersexual fat bodies appears to depend upon the developmental history of gene expression in the cells rather than upon the dose of hormone present in the adult. We will describe experiments using a variety of techniques to measure transcriptional activity of the YP genes in the fat bodies of females, induced males, and intersexual flies. The ultimate aim of the experiments is to understand the mechanism underlying sex-specific expression of YP genes.

MALE MATING ABILITY AS AN IMPORTANT COMPONENT OF FITNESS

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Lewontin (1974, *The Genetic Basis of Evolutionary Change*) states the basic problem of evolutionary genetics to be: How much genetic variation in fitness is there that can be the basis of adaptive evolution? Considering the extensive use of Drosophila in population genetics, it is of great interest to note that work with laboratory populations of *D. melanogaster* in particular, suggests that selection of those males which mate may often be the major influence on gene frequency changes, while differential mating success among male genotypes has been demonstrated in the wild.

A quantitative genetic investigation of competitive male mating ability has been initiated by assaying the effect of inbreeding. Inbred lines produced by full-sib mating have been tested against a standard marked outbred strain for their competitive index (CI♂), as their theoretical inbreeding coefficient (F) increased from 0·25 to nearly 1·0 (complete homozygosity). A continued fall in CI♂ with increasing F has reflected the expectation for a fitness trait, and contradicted a previous investigation (Pendlebury and Kidwell (1974), *Theor. Appl. Genet.*, *44*, 428–42), which used two competing mutant strains, in smaller numbers.

Very recently Brittnacher (*Genetics, 97*, 719–30, 1981) has reported the "virility" of *D. melanogaster* with homozygous second chromosomes to be 56 per cent of that of heterozygous

flies, a greater reduction than we have observed from an "equivalent" amount of inbreeding, again as expected from theory.

COMPENSATION IN SEX RATIO WITH TIME IN *DROSOPHILA MELANOGASTER*

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Experiments were conducted to investigate the instability of the sex ratio with time. Virgin females and males were retained in cultures for seven days. The progeny sex ratio showed a gradual change from excess of females in the first days to an excess of males in later days. The males/total ratio varied from 0·375 to 0·601 in different samples, and was significantly different from 0.5 in 35 per cent of the samples investigated. The overall ratio after one week from the first eclosion was 0·481.

In another experiment, reciprocal 1 : 5 days and 1 : 20 days male:female age were employed with the retention of parents in cultures for seven days. We have been unable to see any influence of age in the first cross. However, the results showed the same changing patterns in sex ratio, and the typical excess of females in the overall result was again found. In all samples of the second cross (1 : 20) the proportions of males were significantly different from 0·5, and a positive correlation between these proportions and time was found.

Results from individual matings with daily transfer of parents to new sets of vials were consistent with the previous experiments, indicating a gradual change in sex ratio over a period of seven days after the first eclosion. Heterogeneity of the sex ratio was tested for each cross. In all cases a significant χ^2 was obtained.

CYTOLOGICAL DEMONSTRATION THAT rRNA GENE EXPRESSION IN BONE-MARROW-DERIVED CELLS IS RELATED TO CELL LINEAGE AND STATE OF DIFFERENTIATION

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The human promyelocytic leukaemia cell line HL-60 can be induced by DMSO to differentiate *in vitro* along the granulocytic pathway. We have applied to this system a standard silver-staining technique which demonstrates active nucleolar organisers on fixed metaphase chromosomes. The results will be discussed in relation to those we have obtained using short-term cultures of normal and leukaemic bone marrow.

VARIABLE DNA SEQUENCES IN FLAX

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When the variety of flax "Stormont Cirrus" is grown under particular environmental conditions, a number of changes are observed in its progeny (Durrant, *Heredity*, *17*, 697, 1962). Among the characters altered are plant height and weight, nuclear DNA content, isozymes of peroxidase, esterase and acid phosphatase and the number of rRNA and 5S RNA cistrons (Cullis, *Heredity*, *38*, 129, 1977; Goldsbrough, Ellis and Cullis, *Nucl. Acid. Res.* in press). The extent of the DNA variation between lines in which differences have been induced (these lines are termed genotrophs) has been determined by renaturation kinetics of the DNA extracted from a number of genotrophs. The data showed that the sequences which varied did not belong to any particular frequency class. Changes were observed in highly repetitive, intermediate and low copy number sequences. A number of cloned nuclear DNA sequences have been isolated and their copy number determined in a number of genotrophs. Three classes of sequences have been identified. (i) Those which do not vary between genotrophs. (ii) Those which do not vary in copy number between genotrophs but appear to

differ in their organisation. (iii) Those which vary in copy number between genotrophs. The sequences which fall into this group appear to vary independently. There was no consistent methylation pattern, as determined by susceptibility to digestion by the restriction enzyme pair NupI/HpaII, observed for either variable or constant sequences.

THE ROLES OF GENES 3·5, 4 AND 13·3 IN PHAGE T1 DNA REPLICATION

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T1 genomic DNA consists of linear duplex molecules with a 6·5 per cent terminal repetition and three cycles of sequence permutation. Intracellular T1 DNA is replicated as linear concatemers of about 3 genome equivalents in length which are packaged into preformed pro-head structures by the excision of headfuls of DNA. The products of genes 3·5 and 4 function in the formation of concatemeric DNA and the product of gene 13·3 plays a role in the conversion of concatemers into DNA headfuls.

The details of these processes, particularly with the permuted phages, are not well understood and we have used nonsense mutations in genes 3·5, 4 and 13·3 to investigate the molecular events in the formation and processing of concatemeric DNA. Our studies indicate that genes 3·5 and 4 code for an exonuclease and that the absence of either gene product prevents the synthesis of concatemeric molecules. The DNA that is made is in the form of stable duplex molecules about 10 per cent shorter than unit length, which are missing terminal sequences. These molecules do not contain the single-stranded gaps present in wild type intracellular DNA and cannot be packed into virions. Gene 3·5 and 4 mutants are also defective in genetic recombination. These phenotypic defects are all restored, albeit partially, by the host's RecE system which codes for exonuclease I.

Normal excision of DNA headfuls from a concatemeric precursor is initiated by cutting at a *specific* packaging (*pac*) site. This is rapidly followed by a sequence *non-specific* headful cutting mechanism which excises unit length genomes. Gene 13·3 codes for a head function responsible for the headful cutting reaction and in the absence of this function the concatemer precursor is slowly degraded by extensive *pac* site cutting.

A MUTANT OF *ESCHERICHIA COLI* K–12 THAT DOES NOT EXPRESS THE *TRA* FUNCTIONS OF THE F FACTOR, AND DOES NOT YIELD GENETIC REARRANGEMENTS WHICH OCCUR IN, OR NEAR THE F FACTOR

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We have been studying genetic rearrangements affecting expression of the *argF* gene of *E. coli* K–12, using the system described below. Strains carrying a *car* mutation are unable to synthesize carbamyl phosphate, an intermediate common to the biosynthesis of arginine and uracil; but if such strains are supplied with high concentrations of citrulline, instead of with arginine and uracil, mutants overproducing ornithine transcarbamylase (OTCase) can synthesize carbamyl phosphate from citrulline by reversing the normal synthesis of cirtulline from ornithine and carbamyl phosphate. In Hfr strains carrying the F factor adjacent to *argF*, and transferring this gene proximally (and also in F-primes derived from such Hfrs), many OTCase-overproducing mutants are unstable; we have already shown that unstable mutations are not recovered when the F-factor is not adjacent to the *argF* gene. We suspect that one of the four insertion sequences in the F-factor (IS-3 γ-δ IS–3 IS–2) may be responsible, at least in part, for the formation of genetic rearrangements which result in over-expression of the *argF* gene product.

We have isolated mutants of our Hfr strain that yield very few unstable mutations. In one of these newly-isolated mutant strains the *tra* functions of the F-factor are not expressed.

MOLECULAR CLONING OF THE *FAS 1* AND *FAS 2* GENES OF *SACCHAROMYCES CEREVISIAE*

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The fatty acid synthetase (FAS) multienzyme complex of *S. cerevisiae*, consisting of two multifunctional polypeptide chains, is encoded by the unlinked genes *fas 1* and *fas 2* (E. Schweizer, *Fatty Acid Synthetase Complexes* in Bisswanger and Schminke-Ott (Ed.) in *Multifunctional Proteins*, Wiley & Sons, Inc. 1980). A cosmid yeast gene bank was constructed using the *in vitro* λ packaging method (Collins and Hohn, *Proc. Natl. Acad. Sci. 78*, 3760, 1981). Since it was desirable to transform both *E. coli* and *S. cerevisiae* directly the cosmid cloning vector used contained the yeast replicon *ars 1*. (Hsiao and Carbon, *Proc. Natl. Acad. Sci. 76*, 3829, 1979).

Eleven clones gave a positive response after *in situ* colony hybridization to probes containing FAS specific sequences. Transformation of four *fas* cosmids into *S. cerevisiae* mutants representing the various complementation groups of *fas 1* and *fas 2* have shown that the *fas 1* gene has been cloned in two of the cosmids and the *fas 2* gene in the other two.

Southern hybridization and genomic plotting have shown that at least 16 Kb of the cloned *fas 1* gene are contiguous on the yeast genome. This result is confirmed by transformation with various sub-clones and physical characterization of the *fas* cosmids. The intact *fas* genes obtained are being investigated by DNA sequencing and deletion mapping for the gene structure corresponding to the domains of the FAS multienzyme complex.

TRANSFORMABILITY OF STRAINS OF *SACCHAROMYCES CEREVISIAE* WITH CHIMERIC PLASMIDS YRp7 AND YEp13 and CLONING OF THE YEAST *PHR* PHOTOREACTIVATION GENE

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Using plasmid YRp7 and selection for *TRP1* , frequencies of transformation (a) per μg DNA and (b) per regenerated spheroplast colony were obtained for (1) a haploid strain yielding high frequency of transformants, (2) a haploid strain yielding a low frequency of transformants, (3) the diploid strain from crossing these 2 haploids and (4) eight segregant strains from 2 tetrads of this diploid strain. Results showed a 1000× difference between strains (1) and (2) calculated by (a) and 100× difference when calculated by (b). The diploid strain was transformed at a frequency lower than strain (2) and it was concluded that high frequency transformation was inherited as a recessive characteristic. All 8 segregant strains showed intermediate levels of transformability indicating polygenic inheritance. Cold treatment of transforming mixtures increased transformation frequencies for some but not all strains.

Using plasmid YEp13 and selection for *LEU2*, 4 related haploid strains gave transformation frequencies of approximately 6000, 500, 0 and 0 per μg DNA. Using a genomic bank constructed in YEp13 and a rapid screening method for photoreactivation of *LEU2* transformants of a *phr* recipient strain, 2 clones assumed to carry the *PHR* gene were isolated. Further investigation of these clones is in progress.

INDUCIBLE DNA REPAIR IN SIMPLE EUKARYOTES

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Evidence will be presented for the existence of a UV inducible DNA survival mechanism in *U. maydis* and *S. cerevisiae*. In both organisms a period of 2–3 hr post-irradiation RNA and protein synthesis is required for maximum cell survival. The induced state is stable for

several hours, as demonstrated by split dose experiments. Studies on mutants defective in DNA excision and recombination repair suggest that the latter, but not the former, forms part of this survival mechanism. An analysis of UV induced mutagenesis in *U. maydis* also suggests that mutagenic repair is involved in induced survival. Attempts to directly demonstrate the *de novo* expression of DNA repair genes following inducing treatments are also described.

CLONING OF THE STRUCTURAL GENE FOR *NEUROSPORA* NADP–SPECIFIC GLUTAMATE DEHYDROGENASE

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A synthetic 17-base DNA sequence (made to match the 5′ end of the mRNA which is known from frameshift analyses) has been used as probe for the *am* gene, coding for NADP–GDH. A 9 kb Hind III fragment which hybridizes strongly with the probe has been cloned in a lambda phage vector. The cloned sequence, labelled by nick-translation, hybridizes to a 9 kb fragment in Southern transfers of Hind III-digested wild type DNA. This fragment is not present in similarly digested DNA from a mutant in which the entire mapped part of the gene has been deleted; the deletion mutant yields instead a more weakly hybridizing 2 kb fragment. The cloned sequence, which seemingly contains at least a major part of the *am* gene, is being further analysed.

A POSSIBLE REGULATORY GENE FOR THE MOLYBDENUM-CONTAINING COFACTOR IN *ASPERGILLUS NIDULANS*

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The synthesis of molybdenum-containing moieties of many molybdoenzymes involves a number of common steps (see reviews in Coughlan (ed.), *Molybdenum and Molybdenum-Containing Enzymes*, Oxford: Pergamon Press, 1980). In the ascomycete fungus *Aspergillus nidulans*, a molybdenum-containing cofactor, whose existence was first postulated by Pateman, Cove, Rever and Roberts (*Nature, 201*, 58, 1964), is essential to nitrate reductase and two distinct purine hydroxylases (formerly designated xanthine dehydrogenases) (reviewed by Cove, *Biol. Rev. 54*, 291, 1979, and Scazzocchio, p. 487 in volume edited by Coughlan cited above). Synthesis of this cofactor requires the integrity of five loci, designated *cnx*ABC, *cnx*E, *cnx*F, *cnx*G and *cnx*H. We have identified a sixth locus, designated *cnx*J, which might be involved in the regulation of cofactor levels. When grown in the presence, but not in the absence, of tungstate or methylammonium *cnx*J$^-$ mutants have reduced levels of the three molybdoenzymes as judged both from growth properties and enzyme determinations. There is no evidence that mutations in *cnx*J can lead to loss of any or all of the molybdoenzymes, but *cnx*J mutations effectively lower the cofactor concentration.

We also report a new cryosensitive *cnx*C$^-$ allele whose phenotype at 37°C (but not at 25°C) shows some similarities to that of *cnx*J$^-$ alleles. Preliminary results suggest a structural role for the *cnx*C (or *cnx*ABC) gene product in the cofactor.

AGROBACTERIUM TUMEFACIENS T–DNA INSERTS INTO THE FLAX GENOME

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Flax (*Linum usitatissimum*) provides an excellent model system for the study of the range and variation in T–DNA insertion following transformation by *Agrobacterium tumefaciens*. The plant forms tumours readily with numerous strains of the tumorigenic bacterium, both

natural isolates and *in vivo* modified derivatives. In addition, flax has a very low nuclear DNA content (1·5 pg) which increases the resolution of detection of single copy DNA sequences.

One particular tumour line induced by *A. tumefaciens* strain T37 has been studied in some detail. It appears to contain 7 normal and one abnormal T-DNA insertions. The limits of the normal insertions conform to those prevously described (Lemmers *et al.* (1980) *J. Mol. Biol. 144*, 353–376) while the abnormal insertion is lacking some 3–4·5 kb of DNA from the right hand end. Digestion of the DNA with Hind III suggests that although the right hand end junction fragments are unique for each insertion, this is not the case for the left hand end where two of the junction fragments appear to be present in multi-copy amounts.

Digestion of the DNA with restriction endonucleases which cannot cut DNA where 5-methyl cytosine is present in the enzyme recognition site shows that several of the inserts are in methylated compartments of the flax genome, the extent of methylation varying from insert to insert.

E. M. RECONSTRUCTIONS OF PACHYTENE NUCLEI IN THE TURBELLARIAN: *MESOSTOMA EHRENBERGII EHRENBERGII*

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At metaphase I during spermatogenesis in *M. e. ehrenbergii* ($2n = 10$), 3 bivalents and 4 univalents form. The bivalents each have a single distally localised chiasma. E.M. serial section reconstructions have shown that 3 short pieces of synaptonemal complex (S.C.) are present in pachytene nuclei in the testis. All the S. C. is contained in a lobe which is separated by an invagination from the main body of the nucleus. The S.C. pieces vary in length from approx. 2 μ to 5 μ, and the greatest amount found in one nucleus was approx. 13 μ. These pieces of S.C. correspond to the paired regions of the bivalents. This system is an example of localised pairing leading to localised chiasmata.

The first meiotic division in oogenesis is achiasmate, but all chromosomes are associated with their homologue and no univalents are present at metaphase I. Preliminary observations on E.M. serial section reconstructions have shown that pachytene nuclei in the ovary contain large amounts of S.C., at least 150 μ. The bivalents appear to be synapsed along their entire length, which is in sharp contrast to the situation present in spermatogenesis.

STUDIES ON THE *ECDYSONELESS*^{1} MUTANT OF *DROSOPHILA MELANOGASTER*

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The temperature-sensitive mutant *ecdysoneless*^{1} (*ecd*^{1}) fails to pupariate when 3rd instar larvae are shifted from 18 to 29°C and this mutant may therefore be defective in some aspect of ecdysteroid biosynthesis at the restrictive temperature. I find that *ecd*^{1} homozygotes fail to eclose when shifted from 18 to 29°C during the first 45 hours after puparium formation. Although metamorphosis occurs, these pharate *ecd*^{1}/*ecd*^{1} adults have defective macrochaetae and genitalia. Adult females are sterile with non-vitellogenic ovaries when shifted from 18 to 29°C from 45 hours after puparium formation. Ecdysteroids may play a role in controlling yolk-protein synthesis in *Drosophila* and the finding that *ecd*^{1} homozygotes are not defective in yolk-protein synthesis at 29°C prompted me to examine ecdysteroid titres during prepupal and pupal development. I find that the ecdysteroid titre, determined by RIA increases and decreases during this period in both Oregon-R wild-type and *ecd*^{1} homozygotes shifted to 29°C at puparium formation. This contrasts with the previously-reported findings of others (Garen *et al.*, 1977: *Proc. Nat. Acad. Sci. 74*, 5099) that an earlier ecdysteroid peak, occurring just before pupariation, is abolished in *ecd*^{1} homozygotes at the restrictive temperature.

EXCISION OF A 412 TRANSPOSABLE ELEMENT OF *DROSOPHILA MELANOGASTER* IN YEAST

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Much of the middle repetitive DNA in *Drosophila melanogaster* is made up of families of mobile elements. The transposable elements 412 are present in 20–40 copies per haploid genome and code for an abundant poly-A^+ RNA. The 412 element is terminally redundant and is flanked by 4 base pair repeats of chromosomal origin. In yeast there is a family of transposable elements known as Ty 1. Ty 1 elements are also terminally redundant and are flanked by 5 base pair repeats of chromosomal origin. The physical similarities between yeast and *Drosophila* transposable elements suggest that 412 transposition may be possible in yeast.

We have developed a system to examine 412 behaviour in yeast. A yeast/*E. coli* replicating plasmid which carries a *Drosophila* 412-containing fragment has been used to transform yeast. In 4 out of 5 transformants there is a loss of 412 from the plasmid. The excision of 412 includes both direct repeats and is not due to a simple recombination event. Data concerning the fate of the 412 element after excision and the transcription activity of this element in yeast will be presented.

THE INDUCER-REACTIVE SYSTEM OF HYBRID DYSGENESIS IN *DROSOPHILA MELANOGASTER*

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F1 females from a cross between males from a class of strains known as inducer (I) and females of the complementary, reactive (R) class show a range of dysgenic traits including partial sterility and high levels of non-disjunction and mutation. This syndrome is the result of interaction between mobile chromosomal I factors and a specific extra-chromosomal condition, reactivity. I factors are thought to be transposable elements, carried on inducer chromosomes. We are analysing a mutant, *white*,^{IR1} isolated from a dysgenic cross and very closely linked genetically to an I factor. We have physically mapped the position of an insertion in the *white* gene, the probable I factor. We are now attempting to clone the I factor by screening a library of DNA from the w^{IR1} strain with a probe for the *white* gene. If we are successful we shall characterise the I factor and other mutants generated by this system.

THE CHROMATIN STRUCTURE OF SPECIFIC GENES IN ERYTHROID CELLS OF *XENOPUS LAEVIS*

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Mature Xenopus erythrocytes are known to be transcriptionally inert. Intact nuclei gently isolated from these cells serve as a poor template for *in vitro* transcription. By contrast, erythroblasts are known to actively synthesize RNA, including transcripts of globin and tRNA genes. In order to develop a model system for the control of gene expression during terminal differentiation, we are investigating the chromatin structure of globin, tRNA and vitellogenin genes in erythroid cells.

As a probe for analysing the conformation of specific genes within chromatin, we are using pancreatic DNase I, as this enzyme seems to preferentially degrade transcriptionally active gene sequences. By following this approach, we are mapping the hypersensitive sites for globin and tRNA genes. We will also report on the effect of exogenous (0·3 M Salt; erythroblast cytoplasm) and endogenous (Ca^{2+} – endonuclease) factors on chromatin digestion.

MUTAGEN SENSITIVITY IN THYMIDINE KINASE DEFICIENT MOUSE LYMPHOMA CELLS

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It has been reported earlier that thymidine kinase deficiency in Friend leukaemia cells of clone 707 results in increased sensitivity to killing and mutagenesis by ultra-violet (UV) irradiation (P. G. McKenna and I. Hickey, *Cell Biology International Reports*, *5*, 555, 1981) and by chemical mutagens (P. G. McKenna and A. A. Yasseen, *3rd Int. Conf. on Environmental Mutagens*, Japan, 1981).

Further experiments have been carried out with two mouse lymphoma cell lines (L5178Y and P388) to determine if thymidine kinase deficiency leads to increased mutagen sensitivity in cell lines other then Friend leukaemia. L5178Y wild-type cells and two thymidine kinase deficient clones L5178Y BU2 and L5178YBU3 were compared for sensitivity to killing by the mutagens, N-methyl-N-nitro-N-nitrosoguanidine (MNNG), ethyl methane sulphonate (EMS), and mitomycin C (MMC). No significant difference in sensitivity was observed between wild type and thymidine kinase deficient cells.

P388 cells and three thymidine kinase deficient clones P388TK2, P388TK3 and P388TK4 were also compared for sensitivity to killing by MNNG, EMS and MMC. Two of the clones P388TK2 and P388TK4 showed greatly enhanced sensitivity to the three mutagens tested.

In the light of these results, and those previously obtained with Friend leukaemia cells, the significance of thymidine kinase in DNA repair processes will be discussed.

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THE EFFECT OF INCREASED MATERNAL AGE ON OOCYTE RADIOSENSITIVITY

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The relative sensitivities of oocytes of young (10–14 week) and old (43–54 week) female mice to radiation-induced chromosome damage have been examined. One-cell embryos recovered from females exposed to various absorbed doses of X-rays have been screened for numerical and structural chromosomes aberrations. In a separate study, the effects of X-irradiation of unfertilized oocytes have been investigated. Results to date indicate an identical response to radiation-induced chromosome damage in oocytes of young and old females, that is, there is no increase in radiosensitivity with advancing maternal age.

DNA REPAIR MECHANISMS IN UNICELLULAR CYANOBACTERIA

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The unicellular species of cyanobacteria, *Synechocystis* PCC 6308 and *Synechococcus* PCC 6301, have been shown to possess extremely efficient mechanisms for the repair of UV-induced DNA damage. These include photoreactivation and dark repair systems. The enzyme involved in the first step of dark excision repair in *Synechocystis*, the UV endonuclease, has been isolated from crude lystates. It shows activity towards UV-irradiated lambda DNA but no activity towards unirradiated DNA. The characterization of this enzyme will be described. The induction and elimination of pyrimidine dimers from the DNA of unicellular cyanobacteria has also been investigated and the implications of these findings to DNA repair studies will be discussed.

ISOLATION OF CHROMOSOME–SPECIFIC HUMAN DNA SEQUENCES BY MOLECULAR CLONING OF HYBRID CELL DNA

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A recombinant DNA library was constructed from a Chinese hamster × human hybrid containing human chromosomes 3, 7, 9, 10, 14, and 22. EcoRI fragments of the hybrid cell DNA were amplified in lambda phage, and those of human origin identified by hybridization of phage plaques to ^{32}P-labelled total human DNA. This assay detects the repetitive elements in human DNA and was shown to discriminate clearly between phage carrying human inserts and those of Chinese hamster origin. The human recombinants were pooled, and, in order to facilitate subsequent manipulation and analysis, the inserts recloned in pAT153.

A selection of recombinant plasmids was digested with a variety of restriction enzymes in an attempt to liberate single-copy sequences. Hybridization of electrophoretically separated plasmid fragments to ^{32}P-labelled total human DNA identified those containing highly repetitive sequences; those fragments not hybridizing to total DNA apparently contain sequences present in 1 to about 20 copies in the human genome. The frequency with which unique sequences were encountered was substantially less than estimated in the literature for fragments of 2 kb or less. Two single-copy probes were examined in detail by hybridizing them to blot-transfers of EcoRI-digested DNA from a hybrid cell panel. One of them recognizes an approximately 3 kb EcoRI fragment of chromosome 3, the other a 6 kb EcoRI fragment of chromosome 7.

THE *BR1A12* VARIEGATED POSITION EFFECT MUTANT OF *ASPERGILLUS NIDULANS*

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Further investigations of this mutant have included demonstration of the autonomy of switching of the variegating *br1A* locus in different nuclear clones in a heterokaryon, studies of phenotypic suppression by 8-azaguanine and other substances and the isolation and characterisation of suppressor and enhancer mutants.

TRANSPOSON–SPECIFIC SITE–SPECIFIC RECOMBINATION *IN VIVO* AND *IN VITRO*

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Transposons of the Tn3 family encode site-specific recombination systems that are used to catalyse the second final step of inter-replicon transposition mediated by these transposons in *Escherichia coli*. In this step a cointegrate molecule containing the donor and recipient replicons separated by two directly repeated copies of the transposon, is converted to separate donor and recipient replicons, each containing a copy of the element. We have been studying both the requirements for and properties of this reaction for several members of the Tn3 family. To date our observations include: 1. Intramolecular transposition of Tn1/3, unlike inter-molecular transposition, occurs independently of the *tnp*R/*res* mediated specific recombination system. 2. *tnp*R protein (resolvase) of Tn1/3 acts preferentially on directly repeated copies of the recombination site (res) within the same molecule. 3. *In vitro* Tn1/3 site specific recombination requires only supercoiled DNA substrate, Mg^{++} and resolvase. 4. To be fully functional, the *res* region must include more than 100 bp in the region of the recombination event.

SEQUENCE–DEPENDENT HELICAL PERIODICITY OF DNA

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We have recently described a new approach to measuring the helical periodicity of random sequence DNA in solution. It consists of binding short, stiff pieces of DNA to various flat surfaces and using DNase I to probe the most accessible phosphodiester bonds. The differences in length of the fragments produced are measured with high precision to give the helical repeat directly. This approach has now been extended to study the periodicity of defined-sequence DNAs using another enzyme, micrococcal nuclease, as well as DNase I. We find the helical repeat of poly (dA-dT) to be $10{\cdot}5 \pm < 0{\cdot}1$ base pairs (bp), a value very close to that found earlier by us for random-sequence DNA, $10{\cdot}6 \pm 0{\cdot}1$ bp, and confirmed here. Poly(dA)·poly(dT) exhibits a distinctly different helical repeat, $10{\cdot}0 \pm 0{\cdot}1$ bp.

THE DYNAMICS OF SEQUENCE TURNOVER AND CONCERTED EVOLUTION OF FAMILIES OF rDNA, HISTONE GENES AND NON-CODING DNA OF *DROSOPHILA*

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Analysis of variation in the organisation of histone and rDNA gene clusters and non-coding families in seven sibling species of the *melanogaster* subgroup has revealed that extensive family homogeneity exists for variant sequences that are diagnostic for each species, (concerted evolution). New sequence variants have been fixed throughout both coding and spacer regions of the histone repeating unit. The homogeneity of rDNA units has involved the fixation of variants within the external non-transcribed spacers and internal transcribed spacers on both the two X and Y chromosome located rDNA clusters. Analysis of individual X and Y nucleolar organisers by genetic manipulation of isofemale lines of small Oklahoma population of *D. melanogaster* has revealed extensive, yet stably inherited, structural polymorphism for rDNA unit length and copy-number. These findings, in conjunction with the observation of length variation within and between the species that is based on the periodicity of an internally repetitious region within the external spacers, suggests that unequal exchange is responsible for a relatively continuous process of turnover of units within the clusters. This turnover has the effect of expanding and contracting the copy number of individual lengths and is probably involved in the accidental replacement of one set of sequences by another. The homogeneity of sequences between clusters on separate chromosomes suggests that the same process, in transferring sequence information from one chromosome to another, could be responsible for the observed homogeneity of variant sequences within a sexual species. Analysis of structural and sequence variation of non-coding high copy-number families shows that such families are also being continually homogenised yet are evolving slowly within the species subgroup. The possible constraints on the relative rates of change of all families will be discussed. Finally the implications for evolutionary genetics of processes of homogenisation of multiple copy families that do not rely on natural selection or drift will be raised.

MOLECULAR GENETICS OF THE MUP GENE COMPLEX OF THE MOUSE

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The major urinary proteins (MUPs) of the mouse are a family of related polypeptides that are synthesized in the liver, secreted, and excreted in the urine. Their synthesis is influenced by oestrogens, androgens, glucocorticoids and growth hormone. MUP mRNA and MUP-specific cDNA hybridize with approximately 20 chromosomal genes, many of which have been cloned and shown to be different. The genes contain at least 6 short exons (total length 1–1·2 kb) and 5 introns (~3 kb). Homologies between flanking regions extend to a total of at least 15 kb.

Most of the MUP genes can be grouped in two main sub-families, each with about 10 genes, which can be distinguished by cross-hybridization and restriction site mapping. The genes of sub-family I contribute most of the MUP mRNA found in mouse liver. Most (and possibly all) of the members of both sub-families are located on chromosome 4. However, no MUP genomic clone has been found to contain either two MUP genes or two copies of a flanking sequence, showing that many (and possibly all) MUP genes are separated by more than 13 kb of intervening DNA.

The urinary MUPs of a single inbred mouse line can be resolved by IEF into about 10 components. The unprocessed *in vitro* translation products of hybrid-selected MUP mRNA resolve into about 20 components. In each case some components are abundant while others are less so. Some are more responsive than others to testosterone induction in the female. The IEF patterns, both of the urinary MUPs and of the unprocessed MUPs synthesized *in vitro*, differ in different mouse strains. An analysis of abundant urinary MUPs in crosses between inbred strains is most simply explained by supposing that some of the structural genes recombine with a fairly high frequency. This would imply that genes of sub-family I are to some extent scattered within chromosome 4.

EVOLUTION OF GLOBIN GENES IN MAN AND THE PRIMATES

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Molecular cloning has been used to analyse in detail the organization of the β-globin gene cluster in a range of primate species. This work is unravelling the complex evolutionary history of this cluster and is revealing the existence of very substantial tracts of highly conserved extragenic DNA potentially involved in the regulation of this multigene family.

AN INSERTION ELEMENT WITHIN INSERTION ELEMENTS

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The type 1 rDNA sequence insertion of *D. melanogaster* is found both in the X chromosome rDNA, and within chromocentral heterochromatin, where it can occur in tandem arrays. One such tandemly arrayed type 1 repeat has been found to contain a 7 kb of "foreign" DNA, which we call the Zeta element. Zeta is estimated to be present in 30–40 copies per haploid genome, distributed in seven major arrangements. We describe the sequence arrangement at the junctions of the Zeta element with the type 1 insert, and the transcriptional characteristics of the Zeta sequence.

SIMULTANEOUS TRANSCRIPTION AND PROCESSING OF RNA FROM MOUSE β MAJOR GLOBIN DNA BY AN *IN VITRO* ENZYME SYSTEM

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A parially purified RNA polymerase prepared from mouse ascites cells transcribes cloned mouse β major globin DNA from a specific initiation point and, furthermore, processes the transcript into discrete size classes. In the case of one template (HS2) the processing occurs at numerous cryptic splice sites throughout the first intervening sequence. However, none of the transcripts corresponds to correctly spliced RNA. Another template (SS) which contains additional 5′ flanking sequences produces a transcript in which the intervening sequence has been spliced out at the correct exon-intron junctions. This activity depends on a DNA sequence present only in the SS DNA and it appears to be exerted in a trans fashion.

THE ORGANIZATION AND EXPRESSION OF THE GLOBIN GENES OF *XENOPUS LAEVIS*

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In *X. laevis*, the major adult α^- and β^- globin genes (α' and β') reside on the same chromosome separated by 8 kb. Two of the minor adult globin genes (α^2 and β^2) are also linked and separated by the same distance. There is no evidence that the major and minor loci are linked and *X. tropicalis*, a diploid relative of the tetraploid *X. laevis*, contains only one α–β locus. These results suggest that there has been gene duplication by tetraploidization and that the major and minor loci reside on separate chromosomes. We have since discovered a major tadpole α-globin gene (αT') 5kb upstream of α' and a minor tadpole α-globin gene (αT^2) 11kb upstream of α^2. This result suggests that an insertion/deletion event between αT and α occurred at some time after the appearance of the major tadpole β-globin, and genomic clones for minor tadpole globins. The locations of these genes relative to the two characterized loci are currently under investigation. The complete nucleotide sequences of α' and β' and their flanking regions have been determined. The start points of transcription have been identified and the conserved *ATA*, *CCAAT*, *AATAAA* and *GT–AG* sequences have been found in the same positions as in other intron-containing genes transcribed by RNA polymerase II. By analysis of nuclear RNA from erythroid cells, full length precursors, and splicing intermediates in their processing, have been detected. Repetitive sequences and RNA polymerase III transcripts have been mapped 5' and 3' of α' and 3' of β'. Nucleotide sequences from the major tadpole α^- and β– globin genes (αT' and βT') will also be presented. We are currently studying the expression of these various genes by microinjection into oocytes and fertilized eggs.

TRANSPOSABLE ELEMENTS IN *DROSOPHILA MELANOGASTER*

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We have been investigating the properties of the 412 family of transposable elements in *D. melanogaster*. In this paper we shall discuss striking similarities between 412 elements and proviruses and the implications this may have for the mechanism of transposition.

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