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The Genetical Society

(Abstracts of Papers presented at the Two Hundred and Second Meeting of the Society on the 1st, 2nd and 3rd April 1985 at the University of Edinburgh)

1. Round table discussion: Genetics education at the school/university interface

Organised by B. W. Bainbridge and A. Radford

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The aim is to stimulate discussion on the teaching of genetics at sixth form and first year university levels. What methods can be used to improve genetics teaching? How can the accuracy of genetics teaching and examining be improved? How will recent progress in genetic engineering and biotechnology influence the genetics contents of the curriculum?

2. Human gene mapping

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The development of somatic cell genetics and, in particular, the use of interspecific somatic cell hybrids, has resulted in an enormous expansion in human gene mapping. Initially, this mapping was carried out by measuring the human gene product of interest and the method was critically dependent upon the use of parental lines for making the hybrids which expressed this gene. The current development of molecular genetics and the use of cloned genes as DNA probes has given rise to a second expansion in human gene mapping, as this technical development circumvents the problems of gene expression. Many genes which are not easily detected at the cellular level can now be mapped. Both methods of mapping will be presented and the technical advantages and problems of each discussed. The current state of the human map will be summarised.

3. Analysis of human DNA repair genes with somatic cell genetics and recombinant DNA techniques

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Genetic analysis of DNA repair processes in mammalian cells has been aided by the "naturally occurring" repair-deficient human syndromes, *e.g.* xeroderma pigmentosum (XP) and by repair deficient rodent cell lines isolated in the laboratory. The genetic complexity of these repair pathways is demonstrated by complementation and gene localisation studies using cell fusion techniques. The data revealed that, for example, excision deficient XP patients can be divided into at least nine complementation groups and that various human chromosomes contain genes coding for DNA-repair enzymes.

The advent of recombinant DNA techniques has provided new approaches to the study of repair genes. Recently we have cloned a human repair gene (ERCC-1) that corrects the repair defect in a CHO mutant cell line. The gene was obtained by transfecting human DNA ligated to a dominant vector into a CHO mutant (43-3B) which is sensitive to UV light and mitomycin-C. The gene was isolated from a cosmid library which was constructed from DNA of a secondary transformant. The repair gene ERCC-1 has a size of about 16 kb. A 900 bp cDNA clone lacking 100–200 bp of the 5′ end of the mRNA was isolated from a human cDNA library. From hybridisation experiments with ERCC-1 using different cDNA probes we identified at least 8 exons. At this moment we are studying in more detail the function of ERCC-1 in DNA repair and its relation to human repair-deficiency syndromes.

DNA-repair processes can be studied also by microinjection. As measured by unscheduled DNA-synthesis the nine excision deficient XP

complementation groups were corrected after injection of crude HeLa extracts or extracts from complementing XP cells. This microinjection techniques is now used for the purification of the protein that corrects the defect in XP cells belonging to complementation group A. The XP-A correcting factor is partly purified and the data obtained so far show that the microinjection assay will be a valuable tool for the purification and characterisation of factors playing a role in mammalian DNA repair.

4. Manipulation of the karyotype of somatic cell hybrids

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The spontaneous loss of human chromosomes from human-rodent hybrids has not only facilitated human gene mapping but, paradoxically, has also limited genetic analysis. In many cases, the chromosome of interest is absent from a crucial set of hybrids or perversely it proves difficult to remove irrelevant chromosomes. These problems complicate gene mapping and obfuscate the genetic analysis of complex phenotypes which may depend on the epigenetic interaction of unlinked genes. We have developed methods for manipulating the human genetic contribution to hybrid cells based on immunological enrichment and biochemical selection techniques.

Human, cell-surface markers defined by monoclonal antibodies can be used when combined with the fluorescence activated cell sorter (FACS) to separate antigen positive and negative subpopulations of hybrid cells. As most human chromosomes encode at least one known cell surface antigen much of the human genome can be manipulated in hybrids by using the FACS. However, this method does not result in the stabilisation of hybrid karyotypes and repeated sorting is often required to maintain the antigen positive phenotype and hence the presence of the desired human chromosome.

For human gene mapping an ideal panel of hybrid cells would be composed of 24 hybrids each with a single, selected, human chromosome. We are attempting to construct this panel by introducing dominant selectable markers at random into the human genome and then using microcell transfer of chromosomes to recipient mouse cells.

A similar strategy is being pursued for fine scale mapping of defined regions of the human genome. Following random integration of dominant selectable markers, chromosome mediated gene transfer results in the production of hybrid cells retaining subchromosomal fragments of human chromosomes. Hybrids containing fragments from defined regions of the human genome can be isolated using specific antibodies and the FACS. A panel of hybrids each containing a different fragment from the same genomic region could be used for final scale mapping.

5. Genetic analysis of tumorigenicity in human cell hybrids

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Previous studies of intraspecies human cell hybrids, derived from the fusions of malignant × normal cells, have shown that the hybrid cells behave as transformed cells in culture but are unable to form tumors in immune deficient mice. Rare tumorigenic segregants arise which have fully regained their tumorigenic potential. Cytogenetic analysis of paired combinations of non-tumorigenic and tumorigenic segregant hybrid cell populations indicated that loss of specific chromosomes are associated with tumorigenic re-expression.

We have now shown by restriction fragment polymorphism (RFLP) analysis that tumorigenic expression of HeLa × fibroblast hybrids is correlated with the loss of at least one copy of chromosome 11 derived from the fibroblast parent.

Methods have been developed to accomplish the selective transfer of specific individual human chromosomes to recipient cells. The transfer of specific individual human chromosomes from normal cells into human cancer cells is currently under investigation.

6. Plant somatic hybridisation

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Plant somatic cell genetics is a relatively new discipline which relies heavily on various plant tissue culture techniques. It is possible to modify the genetic makeup of a single cell, through the use

of cultured cells, and then regenerate an entire plant with the modified phenotype from this modified cell.

Somatic hybridisation by the fusion of plant protoplasts overcomes sexual incompatibility barriers to hybridisation and creates a novel cytoplasmic mix as organelles of both parents come together in a common cytoplasmic milieu after protoplast fusion. Mutants have been hybridised with other mutants to recover somatic hybrid cells and sometimes hybrid plants, by genetic complementation. Double mutants can also be hybridised with wild type species eliminating the necessity for any other selectable marker; and fluorescence activated cell sorting is currently being developed to eliminate the need for the use of mutants for selection. The recent success in transforming plant cells and protoplasts using chimeric plasmids carrying bacterial antibiotic resistance genes is now presenting the opportunity for assessments of limited gene transfer by protoplast fusion.

7. Tissue localisation and chromosomal assignment of a serum protein which tracks the cystic fibrosis gene

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Cystic fibrosis is a high frequency (1 in 2000 Caucasians affected) autosomal recessive disorder. The basic gene defect is probably due to mutation at a single locus which has not yet been identified despite intensive efforts in many centres. Linkage analysis in families has so far failed to assign the gene to a chromosome partly because of difficulties with heterozygote detection. A serum protein, now termed cystic fibrosis antigen, has been identified immunologically (Manson and Brock, *Lancet*, 1, 330-331, 1980) in both affected homozygotes (high level) and in unaffected heterozygotes (low level). By this method antigen is absent in normal serum. We have shown that the protein is present in normal granulocytes. Somatic cell hybrids between a mouse myeloid stem cell line WEHI-3 and human myeloid leukaemia cells, express this protein. Segregation analysis in a series of independent hybrid lines has allowed us to assign the gene for this protein to a single human chromosome.

8. Dominant selectable markers in chromosome mediated gene transfer: An aid to human genome mapping

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The chromosomal insertion of plasmid-borne, dominant selectable markers is a potentially powerful way to isolate previously unselectable regions of the human genome. We tested this strategy as follows. The dominant selectable marker, pSV2-neo which confers resistance to the antibiotic G418, was introduced by DNA/$CaPO_4$ co-precipitation into the human-mouse cell hybrid C121. This hybrid is transformed by virtue of the integration of SV40 genomes into the only human chromosome present, no. 7. G418 resistant clones of C121 were selected and pooled. The number of different chromosomal insertion events present in each pool was estimated following restriction endonuclease digestion and probing of Southern transfers for pSV2-neo. Each pool of independent G418^r clones exhibited a characteristic hybridisation "fingerprint" which was stable on propagation. Mitotic chromosomes prepared from one extensive pool were used to transfect mouse L cells to G418^r. In a screen for the co-transfer of human DNA along with the obligatory pSV2-neo sequences, a single human DNA positive clone was identified amongst 46 G418^r transfectants tested. This transfectant contained a unit of ~200 kb of human DNA but no intact SV40 genomes. It was highly stable on propagation and retrieval from liquid nitrogen storage. These results confirm the potential of chromosome mediated gene transfer with dominant selectable markers as a new and general strategy for the segregation and isolation of any chromosomal region of interest.

9. Novel human transforming gene sequence revealed by chromosome mediated gene transfection

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DNA transfection has shown that the human EJ bladder carcinoma cell line contains an actively transforming c-Ha-ras-1 gene in a 6.6 kb BamHI fragment. Mitotic chromosomes prepared from this cell line were used to transfect the transformation competent C127 mouse embryo fibroblast cell line. The resultant foci of transformed cells were morphologically indistinguishable from each other, as they appeared on the primary transfection dishes. However, significant differences in growth rate and morphology were seen when cloned individually in semi-solid medium. Three morphological types were clearly distinguishable when monolayer cultures were re-established. Each type showed a unique pattern of hybridisation to the c-Ha-ras-1 probe. In one type, the hybridisation was to the usual 6.6 kb BamHI fragment, in the second type only to a fragment of ~11 kb and in the third only to a fragment of ~20 kb. The two transformants containing novel ras-related transforming sequences were no less tumorogenic in immunosuppressed mice than transformants containing the usual c-Ha-ras-1 form. These results suggest that the usefulness of the cellular transformation assay can be extended by using mitotic chromosomes instead of DNA fragments as the donor of potentially transforming sequences. These experiments may be expected to give additional insight into the oncogenic process.

10. The significance of epimutations in somatic cell genetics

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The hypothesis that the methylation of cytosine in DNA is important for the control of gene expression has received considerable support and the pattern of methylation appears to be heritable, through the activity of a maintenance methylase. It has also been demonstrated that treatment with azacytidine (azaC) inhibits methylation and reactivates non-transcribed genes.

Several hamster cell strains with enzyme deficiencies have a very stable phenotype, but experiments in this and other laboratories (Harris, *Cell 29*: 483, 1982; *Somat. Cell. Mol. Genet. 10*: 275 and 615, 1984) show that reversion to wild type can be increased as much as a million fold after treatment with azaC. This strongly suggests that the removal of methyl groups has reactivated an otherwise silent gene and direct evidence for this has been obtained in one case (Clough *et al.*, *Science 216*: 70, 1982). Thus, many strains which are usually regarded as conventional mutants, may have instead a phenotype which is due to an epigenetic control of gene activity.

I propose that heritable changes in gene expression should be referred to as *epimutations*. The CHO strain is thought to have a functionally hemizygous genotype, although the karyotype is near-diploid. This may be due to the presence of active and inactive copies of genes, the latter being methylated and reactivable by azaC. Experiments to test this possibility are in progress.

11. Characterisation of human X chromosomal fragments retained as human/mouse translocations in somatic cell hybrids

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Translocations between human and rodent chromosomes may occur spontaneously in somatic cell hybrids or after treatment with specific agents, *e.g.* X-rays. Without detailed knowledge of the rodent karyotype these complex chromosomes are often difficult to identify and analyse. This report describes the combined applications of cytogenetics, *in situ* hybridisation using ^{3}H-labelled repetitive sequences and Southern blotting to identify and analyse two independent interspecific spontaneous rearrangements involving the human X chromosome in different human-mouse hybrids. Both hybrids were derivatives of clones which originally contained an intact X as the only human chromosome.

One hybrid was found to contain the region Xpter→Xq2(2) translocated on to a mouse chromosome. The other possessed a complex rearrangement in which sequences from two separate X chromosomal regions (the middle of the short arm and the distal part of the long arm) could be detected. The sequences appear to be in one contiguous human chromosomal fragment translocated onto a mouse chromosome. Such hybrids are valuable tools for the isolation and characterisation of probes specific for defined regions of the X chromosome.

12. Expression of variable antigens during the life cycle of African trypanosomes

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The African trypanosomes undergo antigenic variation, in which the parasite's surface coat antigen is replaced by a molecule of different antigenic specificity, rendering inutile the host's specific immune response. The magnitude of antigenic variation prevents attempts at vaccination: each trypanosome can express probably several hundred different variable antigen types (VATs), in an unpredictable and divergent manner. As bloodstream trypanosomes differentiate to procyclic forms in the tsetse fly, antigen expression ceases, returning only when the infective metacyclic stage develops.

Antigen expression by metacyclics displays two remarkable features: the VAT composition of the population is highly predictable and it represents only a small part (perhaps 2 per cent) of the trypanosome's repertoire of VATs. One metacyclic population has been shown to contain just 12 VATs. The predictability is not absolute, as a low level of instability occurs during repeated fly transmissions due to deletion and other rearrangements of M-VAT genes. When metacyclics are injected into a mammal and develop into bloodstream forms, the M-VATs are preferentially expressed and re-expressed for several days until removal by antibody, at which point the VAT originally ingested by the fly is re-expressed by some of the population; the trypanosomes retain a "memory" of the ingested VAT during the procyclic phase when antigen is not synthesised but re-express this VAT only after M-VAT expression. As selective pressure would be expected to operate against predictability in the M-VATs, their preferential expression appears to be a weakness in the strategy of antigenic variation, possibly imposed by the molecular mechanisms of expression.

Each antigen is the product of one gene, from a repertoire of about 1000 genes, which are dispersed throughout the genome, the majority in tandem arrays within chromosomes and a minority at telomeres. Activation occurs at telomeric expression sites. For telomeric genes, this is achieved either *in situ* or by recombinational events occurring between telomeres. For internal genes, it is achieved by the insertion of an extra copy of the gene into an active telomeric site. There are several expression telomeres, of which it appears only one operates at a time. Why are the M-VAT genes expressed preferentially? Analysis of several of them has revealed that they are telomeric and possibly reside in the largest-sized DNA molecules in the genome. Current experiments are investigating genomic location and expression mechanisms.

13. Selective telomere activation and the control of antigen gene expression in trypanosomes

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African trypanosomes escape the immune defence of their mammalian host by changing their antigenic surface coat. Antigenic variation occurs through differential antigen gene activation: only one gene is transcribed at a time among a large collection of specific sequences. This transcription always takes place in a telomere, but it seems that different telomeres can be used alternatively as the gene expression site. Since the trypanosome genome is made up of numerous chromosomes, it would appear that a highly selective process allows the activation of only one telomere at a time. This process seems linked to the differential inactivation of a peculiar telomeric DNA modification system.

Two mechanisms allow antigen genes to be expressed. First, a gene copy can be inserted in the expression site by replacing the formerly expressed gene. This is due to gene conversion whose extent can vary considerably, according to the degree of homology between the recombining partners. This relative extent of homology could be responsible for the differential rate of expression of the antigen types during chronic infection. The second mechanism involves the activation of another telomere with deactivation of the telomere containing the preceeding ELC. This form of activation can occur without apparent DNA rearrangement. The alternative use of these mechanisms leads to rapid changes in the antigen gene repertoire, due to gain and loss of different sequences, and to alteration of their activation rate.

The transcription of antigen genes exhibits several noticeable features. First, like transcription of other genes in trypanosomes, it proceeds discontinuously. A common spliced leader is found in front of many if not all mRNA transcripts, although this spliced leader can be synthesised on a chromosome distinct from that carrying the gene.

Secondly, transcription by trypanosome RNA polymerases is highly resistant to inhibition by the drug α-amanitin, in contrast to other eukaryotic systems. Thirdly, the RNA polymerase transcribing antigen genes can be very rapidly and preferentially released from their templates when trypanosomes are taken up from the blood.

14. Evolution of the repeating epitopes of plasmodia circumsporozoite antigens

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Malaria infections are initiated by the injection of plasmodia sporozoites during the feeding of female mosquitoes. These sporozoites have only one detectable surface antigen, the circumsporozoite protein (C.S. protein). Two fifths of this protein consists of short, repeated segments, the size and sequence of the repeats being subject to at least interspecific variation. The genes encoding these proteins have been isolated from several human, simian and rodent malaria infections. Comparative genomic mapping and DNA sequencing studies indicates that the few hundred nucleotides encoding the repetitive epitopes are rapidly diverging through a mechanism which is not operating in the flanking regions for several thousand kilobases on either side.

15. The molecular genetics of blood stage antigens of malarial parasites

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The protozoan parasite *Plasmodium falciparum*, which causes malaria in Man, has been studied at the genetic level by recombinant DNA techniques. Specific antigens from the blood stages have been defined using monoclonal antibodies and other techniques and their genes cloned and expressed in *Escherichia coli*. Two genes encoding antigens of clinical importance will be discussed (Hall *et al.*, *Nature 311*, 379-382, 1984; Hope *et al. Nucleic Ac. Res. 13*, in the press).

16. Isolation and analysis of nuclear and mitochondrial DNA of the primate malarial parasites *P. knowlesi* and *P. falciparum*

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Restriction analyses and DNA/DNA hybridisation of parasite DNA isolated from monkeys infected with the malarial parasite *Plasmodium knowlesi* has permitted unambiguous identification of the nuclear DNA of this species. Its (G+C) content, as determined by estimations of bouyant density as well as by direct analysis, is about 38 per cent, essentially indistinguishable from that of its primate laboratory host, and grossly different from that of the major human malaria parasite, *P. falciparum*, which has a (G+C) content of *ca.* 19 per cent. In addition, gradient fractionation of total *P. knowlesi* DNA revealed a minor component (*ca.* 1 per cent of the total, with a (G+C) content of about 19 per cent). This DNA comprises covalently closed circular molecules of presumed mitochondrial origin. They have a contour length of about 11.6 μm (*ca.* 35 kb), carry a small cruciform structure and have six HindIII restriction sites. A very similar set of restriction fragments has been observed in HindIII digests a total *P. falciparum* DNA probed with the *P. knowlesi* circles. These also summate to about 35 kb and circular molecules of appropriate size have been observed in Kleinschmidt spreads of *P. falciparum* DNA. It appears that the mit DNAs of these two species are more closely related than are their nuclear DNAs.

17. Cloning an *Aspergillus* developmental gene by transformation

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We have developed a transformation system for *Aspergillus nidulans* using a vector containing the selective marker *argB*$^{+}$, and with it a frequency of transformation of 500 stable transformants/μg plasmid DNA can regularly be achieved. The

evidence suggests that transformation is by integration, but spontaneous excision of integrated plasmids is apparently frequent enough to allow the recovery of transforming plasmids in *E. coli*.

This frequency of transformation and the ability to recover transforming plasmids has allowed us to screen a gene bank constructed in the *argB* vector from which we were able to isolate and clone the *A. nidulans* developmental gene *brlA* by visual selection. We have shown by mapping the functional *argB* allele of *brlA* transformants that a plasmid containing the *brlA* gene and *argB* for selection usually integrates at the chromosomal *brlA* locus.

18. Polygenic control of gene conversion in *Ascobolus immersus*

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Gene conversion frequency is under the control of genetically heritable factors; there is also evidence that the environment plays a role. In *Ascobolus immersus* it is known that at least three discrete oligogenes control the frequency and characteristics of gene conversion at the locus *wI* coding for the colour of ascospores (Helmi and Lamb, *Genetics 104*, 23, 1983). An investigation of the effects of selecting "high" or "low" frequencies of gene conversion has revealed polygenic control coexisting with the already known conversion control factors (*ccfs*). The effects of these polygenes on the *wI* locus, as well as on unlinked genes coding for spore colour, have been studied.

Until now the polygenic control of gene conversion has been neglected and it is here shown to be of at least an equal importance to the major genes studies and could play a significant part in the unmasking of the shrouded processes involved in recombination.

19. The use of human-rodent somatic cell hybrids in gene mapping on chromosome 19

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In order to localise unique DNA sequences to particular chromosomes a panel of rodent-human somatic cell hybrids has been constructed. These hybrids also contain chromosomes derived from individuals with balanced reciprocal translocations involving chromosome 19 and therefore provide a means of localising sequences to various parts of this chromosome.

A second independent series of hybrids (WILF) has been obtained in which 19 appears to have fragmented spontaneously, thus providing a number of other breakpoints along the chromosome. These lines have been examined cytogenetically using *in situ* hybridisation. They appear to contain little human material other than chromosome 19. A library has been constructed using DNA from one of these lines and used as a source of chromosome 19 probes. The usefulness of this library in studies to find markers closely linked to myotonic dystrophy on chromosome 19 will be discussed.

20. Using somatic cell genetic techniques to analyse the chromosome 11 short arm deletions associated with the Aniridia Wilms' Tumour (AWT) syndrome

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Constitutional chromosomes 11p deletions from five patients with aniridia (two with AWT) have been immortalised by Epstein-Barr virus transformation of peripheral lymphocytes. The normal and deleted chromosomes from three patients have been segregated into mouse-human cell hybrids. The previously cytologically defined deletions have been analysed with available chromosome 11-assigned DNA probes for β-globin, c-Ha-ras-1, parathyroid hormone, insulin, calcitonin and apolipoprotein A1. In addition we isolated and used a cDNA probe for the enzyme catalase which was previously assigned to the 11p13 region known to be involved in most of the deletions described in AWT. Another linked marker, lactate dehydrogenase, was analysed by enzyme electrophoresis. Using the information gained from cell hybrids, we are developing techniques to allow

direct analysis of deleted genes in lymphoblastoid cell DNA by dosage measurements. We are also using somatic cell genetic techniques to isolate and clone out the whole of the 11p13 region which is common to all the deletions.

21. Detection of unique DNA sequence in extracts of chromosome mediated gene transfectants: An aid to chromosome mapping

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We describe a method for the rapid and quantitative blotting of DNA from mammalian cell extracts. The relative contributions of human and mouse DNA in hybrid or transfected cells can be accurately determined within 24 hours of achieving $\sim 10^6$ cells using species-specific probes to repeated DNA sequences. The presence or absence of single-copy DNA sequences in extracts from $\sim 5 \times 10^6$ can be reliably determined within a single sub-culturing period.

Mitotic chromosomes prepared from a human EJ bladder carcinoma cell line were used to transfect the transformation-competent, mouse embryo fibroblast cell line C127. Transformed foci were picked and the method used to quantitate the total amount of human DNA transferred in each case. Each transformant showed hybridisation to the oncogene on human chromosome no. 11, c-Ea-ras-1. In one transformant, shown to contain the equivalent of 15-20 per cent of a human chromosome no. 11, the co-transfer of the syntenic marker β-globin was demonstrated. This was confirmed by subsequent hybridisation analysis of restricted DNA in Southern transfers. The cell extract DNA blotting procedure thus fulfills all the requirements for a primary chromosome mapping strategy limited only by the availability of species-specific probes for repeated DNA sequences and the single-copy marker sequences of interest. It also confirms the usefulness of chromosome mediated gene transfer as a strategy for the subchromosomal dissection and genetic analysis of the human genome.

22. Isolation and preliminary analysis of cell hybrids retaining translocations from female Duchenne Muscular Dystrophy patients

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The locus for Duchenne Muscular Dystrophy has been assigned to the X-chromosome short arm by the discovery that several girls suffering from DMD also carry *de novo* X: autosome translocations with breakpoints in the band Xp 21, (Verellen-Dumoulin *et al., Human Genetics*, in press). We have established both cell lines and somatic cell hybrids from some of these patients in order to undertake a detailed molecular study of these translocation breakpoints. Hybrid clones have also been established from cells of individuals with X:autosomal translocations with different breakpoints in Xp.

Relevant hybrid clones have been examined cytogenetically and for the presence of various biochemical markers. DNA preparations have been analysed with a variety of X-chromosomal probes to verify the content of X-chromosomal fragments. Where necessary, hybrids have been recloned or back selected in 6-thioguanine to achieve representation of desired X-chromosomal segments.

The hybrid panel is currently being used to sub-regional assignment of Xp chromosomal sequences isolated in this and other laboratories. It will be of value in the physical mapping and ordering of both established and novel probes of potential value in linkage studies involving the DMD locus as well as in allowing comparisons between the various Duchenne translocations.

23. Control of nuclear division in hybrids between normal and transformed cells

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Cytochalasin B (CB) inhibits cytoplasmic cleavage in animal cell lines. In its presence normal cells

remain mononucleate or binucleate. Transformed cell lines continue DNA synthesis and nuclear division after the arrest of cell division resulting in the production of multinucleate cells. This phenomenon has been described as uncontrolled nuclear division (O'Neill, *Cancer Research 34*, 1070, 1974). There is evidence that such loss of control of nuclear division in transformed cells is a reflection of their independence of growth factors for the initiation of DNA synthesis.

We have examined hybrids between pairs of cell lines one of which displayed multinucleation in the presence of CB and the other did not. In 6 such crosses the hybrids failed to show multinucleation in CB. In one set of hybrids segregant sub-clones were isolated in which nuclear division was uncontrolled. These had undergone chromosome loss.

In a series of fusions between the mouse cell lines A9, which has uncontrolled nuclear division, and 3T3, which has controlled nuclear division, suppression of uncontrolled nuclear division was observed to occur but only transiently. In 20 hybrid clones examined, loss of control of nuclear division was re-expressed within 20-30 days after fusion. This change in phenotype was not accompanied by any significant change in chromosome number.

It is concluded that as with several other aspects of the transformed phenotype, loss of control of nuclear division is essentially recessive to the normal phenotype. In some cases it may, however, be under more complex genetic or epigenetic control.

24. Molecular analysis of mouse MHC genes

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A review will be given about our current knowledge on the structural organisation of the genes of the major histocompatibility complex of the mouse. Experiments will be presented which involve the isolation of MHC genes from mouse DNA libraries constructed in cosmid vectors and the isolation of overlapping clones by the techniques of chromosome walking. The goal of these experiments is to completely characterise the MHC at the molecular level, to search for genes so far not allocated to the MHC and to map unusual genetic elements, like recombinational hot spots and hypervariable chromosomal regions encoding the highly polymorphic MHC molecules.

25. The Qa and TL series of antigens

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The *Q* and *TL* subregions of the major histocompatibility complex (MHC) of the mouse determines a series of class I molecules represented on cells of the hematopoetic system. The *Q* subregion determines the Qa-2, Qa-3, Qa-5, Qa-6, Qa-7, Qa-8 and Qa-9 antigens while the *TL* subregion determines the Qa-1, Qa-6, Qa-11 and TL antigens. At least three of these antigens, Qa-1, Qa-2 and TL, are known to be present on class I molecules.

The Qa-2 molecule appears to be rather unique among known class I molecules. It lacks the high degree of polymorphism exhibited by other class I molecules; no structural polymorphism was detected even among *Mus* subspecies.

We have used the approach of examining the *Q* subregion RFLP's in mutant vs. wild type mice to identify the *Qa-2* gene(s). The mutants in this case are sublines of BALB/c that have lost their capacity to express detectable Qa-2 family products on the lymphocyte surface. Our results indicate that the mutant mice have an extensive deletion in this region.

26. Evolution of t and H-2 complexes in the mouse

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The short chromosome No. 17 of the house mouse carries two complexes of loci, one (t) having a pleiotropic effect on a number of traits (segregation of chromosomes at meiosis, fertility, frequency of crossing over, embryonic development) and the other (H-2) coding for molecules that provide the context for the recognition of foreign antigens by thymus-derived lymphocytes in the initial phase of the immune response. We have isolated from wild mice a large number of chromosomes which carry special constellations of *t* and *H-2* loci. All these chromosomes appear to derive from a single ancestral chromosome that existed among wild mice before they split into the *Mus musculus* and *M. domesticus* species. A combination of genetic, serological and molecular approaches allowed us

to trace the evolution of these *t-H-2* chromosomes. The implications that can be drawn from this evolution will be discussed.

27. The biology and clinical importance of the HLA system

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An overview will be given of studies that indicate that HLA alleles are associated with survival during epidemics. These findings will be discussed in the light of the well-known association between HLA and various (auto-immune) diseases. The potential usefulness of the molecular genetics of HLA will be discussed in relation to the diagnosis and pathogenesis of HLA-associated diseases. Finally, a brief update will be given on our experience with HLA matching for organ transplantation.

28. Cell interactions and the MHC

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Experimental work carried out in this laboratory on the role of the MHC in cell interactions will be described. Similar work from other laboratories will be reviewed. Particular attention will be given to MHC effects on adhesion of endothelia, lymphocytes, fibroblasts and epithelia to themselves and to other cell types as well as to non-living surfaces. The roles of MHC fragments in the media and of MHC antigens derivatised to non-living surfaces will be reported. Attention will be given to both Class I and Class II effects. The effects on more complex cell behavioural reactions will be discussed. The results will also be related to ideas on CTL interactions with their target cells.

29. Materno-fetal aspects of MHC

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A review will be given on our current knowledge of the expression of MHC antigens and other major antigens on the surface of trophoblast. Experiments will be presented on the isolation of trophoblast cells (of fetal origin) and their sensitivity to attack by anti-MHC and by immune cells.

30. Fertility in female XO mice- A reflection of prophase pairing?

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XO female mice are known to have a shortened reproductive life span (approximately one third compared to normal controls). This defect has been attributed to a reduced oocyte pool, which is utilised at a rate comparable with normal females. The origins of such a reduction in oocyte number has been studied by Burgoyne and Baker (in "Development and Function of Reproductive Organs", Int. Congr. Series 559, 122, 1981) who have shown that no germ cell loss is seen up to day 18.5 of gestation, although the number of degenerating pachytenes (Z-cells) was increased over the numbers found in normal females. They further speculate that some form of meiotic pairing failure could be operating. To further examine this situation, synapsis at the synaptonemal complex level was examined with the electron-microscope over the 16–20 day period of gestation in female XO mouse foetuses. The results show that at an early stage of prophase (day 16) the single X chromosome in a small percentage of cells attempts to satisfy pairing requirements by initiating either self pairing or pairing with other autosomal complexes. By day 19 various degrees of X chromosome pairing are observed (in up to 50 per cent of all oocytes), all of which represent non-homologous synapsis. It may be that those cells in which X pairing is most complete contribute to the final oocyte pool, while those in which least or no X chromosome synapsis occurs degenerate, leading to the reduced numbers within such a pool.

31. Sequence divergence and allelism in the class 1 HLA gene family

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The genetic basis underlying the extreme polymorphism of the classical transplantation loci HLA-A, HLA-B and HLA-C in Man has been an enduring enigma. In an attempt to understand their molecular evolution we have established the complete nucleotide sequences of three functional class 1 HLA genes, HLA-A3 (Strachan *et al.*, *EMBO J. 3*, 887, 1984), HLA-CW3 (Sodoyer *et al.*, *EMBO J. 3*, 879, 1984) and HLA-A24 (Nguyen *et al.*, *Immunogenetics*, in press). This data base together with equivalent data recently obtained from the HLA-A2 and HLA-B7 genes (Keller and Orr, *J. Immunol.*, in press; Biro *et al.* Cold Spring Harbor Symp. Quant. Biol. *47*, 1079, 1983; S. Weissman, pers. commun.) permits a detailed sequence comparison of allelic genes at the HLA-A locus and of non-allelic genes at all three classical transplantation loci. The results show that allelism exists at the nucleotide level: sequence homology between non-allelic genes is significantly less than between allelic genes, with locus specificity being most pronounced at the 3′ end of the genes. Nucleotide differences between non-allelic genes show a non-random pattern of distribution with pronounced clustering of mutations in the exons representing the first two extracellular domains α_1 and α_2, and also at the start of the exon encoding the third extracellular domain α_3. Analysis of the frequency of nucleotide substitution at the 1st, 2nd and 3rd base positions of codons reveal that in all cases there is a large excess of 3rd position changes in the exon encloding α_3, which is consistent with a strong conservative selection pressure. In contrast, the overall frequencies of substitution at the 3 base positions in the exon encoding α_1 and also the exon encoding α_2 are approximately equal which could indicate a passive process of almost complete relaxation of conservative selection pressure. However the extreme clustering of 1st and 2nd position substitutions within these exons is inconsistent with a passive process of unconstrained mutation and strongly indicates a positive selection pressure for sequence replacement. Evidence for the role of gene conversion as a generator of sequence variation is presented.

32. Structure and expression of mouse major histocompatibility complex class I genes encoding Qa2 reactive polypeptide

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Two studies have been performed on the class I genes of the mouse major histocompatibility complex in order to identify class 1 genes which encode Qa2 reactive polypeptides. In the first study a deletion of DNA in the BALB/c BY ($Qa2^-$) Qa region has been mapped using restriction enzyme digestion, Southern blotting and hybridisation to three DNA probes. The deletion extends from the 3′ end of the Q6 gene to the 5′ end of the Q7 gene (B10 gene nomenclature) and probably has resulted in the generation of a fusion gene. The second approach was to identify Qa2 reactive polypeptides in L cells transfected with each of the Qa region genes (Q1–Q10). Using immunoprecipitation of radiolabelled polypeptides Qa2 reactive polypeptides were found only in Q6, Q7, Q8 and Q9 transfected cells. The BALB/cJ mouse Q6 region is very similar to that of the B10 mouse except that the Q8 and Q9 genes are fused to give a single gene. It is proposed that in BALB/cBY a similar fusion has taken place between the Q6 and Q7 genes and that mouse is $Qa2^-$ because all genes in the Q6 to Q9 region are structurally altered by gene fusion events.