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THE GENETICAL SOCIETY

(Abstracts of papers presented at the TWO HUNDRED AND FIFTH MEETING of the Society held on Friday, 14th and Saturday, 15th November 1986 at UNIVERSITY COLLEGE, LONDON)

1. Selection of somatic cell hybrids with specific chromosome content for mapping the WAGR syndrome

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WAGR (Wilms tumour, aniridia, genitourinary abnormalities and mental retardation) syndrome is frequently associated with deletions on the short arm of chromosome 11. The deletions vary in size but always include part of band 11p13. To home in on the Wilms tumour and aniridia loci the end points of the different deletion breakpoints need to be defined at the DNA level. For unambiguous results the deleted and normal chromosomes 11 from a number of patients need to be segregated from each other, and analysed using as many cloned probes for the region as possible. The probes themselves can be selected from libraries produced from cell hybrids containing only appropriate parts of chromosome 11. The availability of species-specific monoclonal antibodies directed to cell surface markers encoded by genes on chromosome 11 has allowed us to use the fluorescence activated cell sorter (FACS) to select for a large number of hybrid cells with the appropriate chromosome content. These hybrid cells have been invaluable tools for moving toward the Wilms tumour and aniridia loci.

2. Mapping of gene specific DNA markers on chromosome 11 to define the WAGR region

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Cloned probes for a number of available chromosome 11 assigned genes were used to define the extent of deletions associated with the Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation (WAGR) syndrome. Establishing reliable dosage studies for a number of different probes has proved difficult. We have therefore concentrated on segregating the deleted chromosome 11 from a number of patients in somatic cell hybrids and analysing DNA from these to produce a consistent map of chromosome 11p. At the same time we have determined the deletion breakpoints at a molecular level and shown that the results are compatible with all the deletions studied having arisen as a simple interstitial loss of a contiguous region of the chromosome.

3. Restriction fragment length polymorphisms (RFLPs) in the region of the human estrogen receptor gene

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Human breast cancer is a complex of neoplastic diseases that vary in histological appearance and biological behaviour such as potential for invasiveness and metastasis. In approximately 40 per cent of human breast cancers, a higher than normal

level of estrogen receptor (ER) is present. The development of the majority of the cancers of this type is blocked by treatment with antiestrogens thereby implying that high levels of ER are necessary for this process. At present, there are no explanations at the molecular level for the different levels of ER which are found in different tumours. As an initial step towards a greater understanding of these mechanisms, we have initiated an analysis of the human ER gene locus. The experiments we report consist of the digestion of panels of 10 normal DNAs, 10 breast cancer tumour DNAs and DNA from the cell lines MCF7 and T47D with the enzymes *EcoR*I, *Sst*I, *Msp*I or *Tag*I, followed by Southern blotting and hybridization with radioactive probes (obtained from P. Chambon) which correspond to the 5′ or 3′ portion of the ER. To date, 4 RFLPs have been found in the *EcoR*I digestions, 2 with *Msp*I, 2 with *Taq*I and one with *Sst*I. Although some of these were peculiar to tumour samples, we have no evidence yet which correlates the RFLPs with an abnormal level of ER or with tumour formation.

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4. Amplification of c-Ki-ras in testicular teratomas

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Teratomas are tumours composed of multiple tissues foreign to the part of the body, usually the gonads, where they occur. Testicular teratomas originate from primordial germ cells. There is evidence that they are characterised by cytogenetic abnormality, particularly formation of one or multiple copies of a putative isochromosome 12p (Delozier-Blanchet *et al.*, *Cancer Genet. Cytogenet.*, *15*, 375, 1985; Atkin and Baker, *Cancer Genet. Cytogenet.*, *10*, 199, 1983). The c-Ki-ras oncogene has been mapped to chromosome 12p (Popescu *et al.*, *Somatic Cell and Molecular Genet.*, *11*, 149, 1981). Southern blotting of genomic DNA from a solid tumour and a tumour cell line detects amplification of this sequence. By autoradiography and scanning densitometry this is estimated to be between five and eight fold. These data support the proposal that the small marker isochromosome observed in these teratomas is at least in part derived from chromosome 12p.

5. Molecular genetic investigations in colorectal cancer

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Investigations are being carried out on a series of 20 colorectal carcinomas and cell lines. Transfection of N1H3T3 cells with DNA from 17 tumour samples and 3 cell lines has proved positive in 6 cases; 3 have so far been shown to contain human sequences and are being probed for 'ras' and other oncogenes. Approximately 10-fold amplification of K-ras sequences has been found in two tumours. Restriction enzyme digestion was employed to detect changes at the 61st codon of K-ras; no positive results were found in 15 carcinomas.

In order to identify the regions of the human genome involved in the development of colorectal cancer, we are searching for loss of genetic markers in carcinoma samples by comparison with normal tissue from the same individual. Eleven tumours were investigated using the 10 most polymorphic enzyme systems; 3 showed loss of gene expression. Changes are also being monitored by the use of polymorphic DNA probes; testing of 8 tumours with 5 probes has revealed loss of one restriction fragment to date.

Results are being related to clinical staging where this is known.

6. Regional mapping studies on chromosome 19

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Chromosome 19 is currently the best mapped of the smaller human chromosomes. More than 30 distinct gene loci, including disease loci, red cell enzymes, serum proteins and blood groups have been assigned, together with a number of anonymous DNA segments. A large linkage group, spanning virtually the whole length of chromosome 19 has been defined and recently described (Shaw *et al.*, *J. Med. Genet.*, *23*, 2, 1986). Using only family data, Sherman, Ball and Robson (*Ann. Hum. Genet.*, *49*, 181, 1985) suggested a

possible order *LDLR-LE-C3-LW-PEPD-DM-[Se, H]-APOC2-APOE-LU* with LDLR the most distal marker on the short arm.

We have used a variety of gene mapping methods to obtain a regional localisation for a number of gene loci on chromosome 19. Human-hamster somatic cell hybrids have been constructed using lymphocytes from individuals carrying balanced translocations involving chromosome 19, with breakpoints at 19p13·2, 19cen, 19q13·1 and 19q13·3. The human chromosome content of these hybrids has been determined using biochemical and DNA markers, and karyotyping by G and G-11 banding. A panel of hybrids has been obtained which contain the translocation products of chromosome 19 as the only region of chromosome 19 present.

Family studies using two point lod score analyses are also being carried out on families segregating for *PEPD* and other polymorphic markers on chromosome 19. Data obtained so far have shown *PEPD* to be closely linked to *CYP1* in males, with a maximum lod score of +2·69 at $\theta = 0{\cdot}01$. The hybrid and family data combined have provided a revised map of chromosome 19.

7. Cell killing and mutagenesis in wild-type and deoxycytidine kinase-deficient Friend leukaemia cells

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Wild-type (clone 707) Friend leukaemia cells and two deoxycytidine kinase-deficient (DCK^-) subclones were compared for sensitivity to cell killing and mutagenesis following exposure to ultra-violet irradiation (UV), ethyl methane sulphonate (EMS) and methyl methane sulphonate (MMS). Four UV doses were utilized; 1·2, 2·4, 3·6 and 4·8 J m^{-2}. In the case of chemical mutagens, three 16-hour doses of EMS (100, 200 and 300 hour μg ml^{-1}) and four 16-hour doses of MMS (5, 10, 15 and 20 μg ml^{-1}) were utilized. A clear dose-related response was observed for both cell killing and mutagenesis (to 6-thiguanine resistance) with each mutagen and each cell type. There was no clear pattern of altered mutagen-sensitivity in the DCK^- subclones relative to the wild-type cells. This implies that in this cell line, DCK does not play a key role in those events significant for the normal functioning of DNA repair processes.

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8. Linkage and dominant spinocerebellar ataxia

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Autosomal dominant inheritance has been demonstrated for a group of conditions referred to as spinocerebellar ataxia (SCA). SCA however may comprise an heterogeneous group, in some of which there is evidence for linkage with the major histocompatibility complex (HLA), on the short arm of chromosome 6.

We are studying seven families with this disorder and are using a range of recombinant DNA probes from the short arm of chromosome 6 to detect linkage and to identify the sites of recombination in this region. Preliminary data suggest that the locus for the HLA linked subgroup of SCA lies distal to the HLA locus.

9. Cystic fibrosis (CF) antigen: an alternative approach to the basic defect in CF

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CF antigen is a 12,000 Mwt protein found to be elevated in the serum of CF patients and of clinically normal obligate heterozygotes. The protein is synthesised in normal peripheral granulocytes and in chronic myeloid leukaemia (CML) cells. The gene for CF antigen has been assigned to chromosome 1 by analysis of somatic cell hybrids capable of expressing this differentiated function (van Heyningen *et al.*, *Nature*, *315*, 513, 1985). It

is not therefore the product of the aberrant CF gene on chromosome 7. Knowledge of its identity and biological function may, however, give clues to the nature of the basic defect. We have produced monoclonal antibodies to CF antigen (Hayward *et al.*, *J. Immunol. Methods*, *91*, 117, 1986). These have been used for immunopurification of the protein. Partial amino acid sequencing permitted the synthesis of an oligonucleotide probe which was used to isolate a cDNA clone from a λgt10 CML cell cDNA library. Sequencing of this clone revealed that CF antigen has strong homology with intestinal and brain calcium binding proteins. The basic defect in CF is now thought to be at the level of control of chloride channel activity in exocrine glands. Calcium binding proteins participate in signal transduction in such control pathways.

10. Localisation of chromosome breakpoints in ataxia telangiectasia lymphocytes using *in situ* hybridisation

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Various chromosomal translocations have been described in peripheral lymphocytes from patients with ataxia telangiectasia often involving the sites of the immunoglobulin superfamily genes (Aurias *et al.*, *Hum. Genet.*, *72*, 210, 1986). Clones of cells with some of these translocations may appear in the blood, most being very small. We have studied two large non-leukaemic clones (70 per cent of T cells) with translocations *t*(14; 14)(q11; q32) and *t*(X; 14)(q28; q11) respectively. Using *in situ* hybridisation of probes on the *t*(14; 14) clone we have shown that the 14q32 break-point lies outside the IgH locus and proximal to it with respect to the centromere. The 14q11–14qter segment of the homologous 14 carrying the constant gene region of the T cell receptor α chain locus is translocated to this 14q32 site (Kennaugh *et al.*, *Hum. Genet.*, *73*, 254, 1986). In the *t*(X; 14) the constant region of the α chain locus is translocated to Xq28 and although a reciprocal translocation is likely from X to 14, the break-point appears distal to the site of the RFLP St14 and to the G-6-PD gene.

Both clones therefore appear to involve translocations of a single T cell receptor gene to the site of an unidentified gene. Involvement of the α chain gene may be required for successful non-malignant T cell proliferation. Further genetic change is required for transformation to full malignancy.

11. A cDNA clone coding for human sucrase-isomaltase

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Sucrase-isomaltase is one of a family of enzymes found in jejunal microvillar membranes. The restricted tissue distribution of this enzyme has up to now precluded conventional genetic analysis by family studies or by use of somatic cell hybrids and much of the biochemistry of sucrase-isomaltase has been elucidated using animal models such as the rat and rabbit. In order to investigate the biochemical genetics of sucrase-isomaltase in man we have made use of monoclonal antibodies both to study the protein in human post-mortem tissues and to isolate a cDNA clone from a λgt11 expression library.

The recombinant cDNA encodes about half the human sucrase/isomaltase protein and has been used in preliminary studies of the sucrase-isomaltase mRNA. It has also allowed the chromosomal assignment of the human sucrase-isomaltase gene and since it recognises more than one restriction fragment length polymorphism in human DNA more precise localisation by family studies should be possible.

12. Detection of β-thalassemia mutations occurring in different DNA haplotypes in Greece by the use of synthetic DNA probes

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We have recently determined the range of DNA haplotypes occurring in a sample of 69 β-thalassemia patients in S. W. Greece and estimated their relative frequencies. β-thalassemia mutations have been shown (Orkin *et al.*, *Nature*, *296*, 627,

1982) to be linked to specific DNA haplotypes and this finding has been used in the prenatal diagnosis of β-thalassemia by restriction enzyme analysis of fetal DNA. In Greece the majority of the β-thalassemia mutations are single base substitutions which are not directly detectable by cloned DNA probes. We report here the results we have obtained on the delineation of the β-thalassemia mutations and haplotypes occurring in our sample of patients, by the use of synthetic DNA probes. Four synthetic oligoprobes (nonadecamers) were used each of them detecting one of the following mutations: (1) β^+ IVS-1 110, (2) β°-39 nonsense mutation, (3) IVS-1 1(G-A) frame-shift (β°) and (4) IVS-1 6(β^+). (Oligoprobe hybridisations were carried out as described by Pirastu M. *et al.*, *N. Eng. J. Med.*, *309*, 284, 1983). *We* have checked in total 52 β-thalassemia chromosomes of haplotypes I, II, V, VI and IX and it was found that haplotype I carries in 81 per cent of the cases the mutation β^+-110; haplotypes II and IX carry mutation β°-39 in 85 per cent of the cases; haplotypes V and VI were 100 per cent linked to mutations IVs-1 1 and IVS-1 6 respectively. These five haplotypes represent the 89 per cent of all haplotypes detected in 138 β thalassemia chromosomes analysed in a previous study. It is apparent that detection of the corresponding mutations with synthetic DNA probes is of particular importance for the prenatal diagnosis of the condition in Greece. Our results also contribute to the basis for offering first trimester prenatal diagnosis to childless couples, something that is not possible by haplotype linkage and analysis.

13. Increased sensitivity to cell killing and cytogenetic aberrations in thymidine kinase-deficient Friend leukaemia cells following gamma-radiation

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Deficiency of thymidine kinase in Friend leukaemia cells leads to increased sensitivity to cell killing and mutagenesis by UV-irradiation (McKenna and Hickey, *Cell. Biol. Int. Reps.*, *5*, 555, 1981), chemical mutagens (McKenna and Yasseen, *Genet. Res.*, *40*, 207, 1982) and enhances the frequency of mitomycin C-induced cytogenetic aberrations (McKenna and Yasseen, *Leukem. Res.*, *9*, 501, 1985).

Wild-type Friend leukaemia (clone 707) cells and two thymidine kinase-deficient subclones 707BUE and 707BUF, having thymidine kinase activities of 1·4 and 0·7 per cent that of wild-type cells respectively (McKenna and McKelvey, *Somatic Cell Mol. Genet.*, *12*, 325, 1986), were compared for sensitivity to killing and the induction of cytogenetic aberrations following gamma-irradiation. Three doses of gamma-irradiation were used; 150, 300, and 450 cGys, and cells were harvested for metaphase spreads after 4, 8, 12, 15, 29 and 43 hours. Increased sensitivity to the induction of cytogenetic aberrations for all three doses of gamma-irradiation used was apparent in the two thymidine kinase-deficient subclones at 15, 29 and 43 hours. Considerably increased sensitivity to gamma-radiation-induced cell killing was also observed in the two thymidine kinase-deficient subclones relative to wild-type cells. Subclone 707BUE, having twice the thymidine kinase activity of subclone 707BUF, consistently exhibited greater resistance to gamma-irradiation than did the lower thymidine kinase activity subclone 707BUF.

In the light of these and earlier reported results the significance of thymidine kinase for accurate DNA repair is discussed.

14. Redefining the components of gene conversion

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In the past it has been possible to express gene conversion in terms of an algebraic model where the event is broken down into its component parts such as γ, the probability of hybrid DNA forming between two non-sister chromatids (Kalogeropoulos and Thuriaux, *Genet. Res. Camb.*, *40*, 1, 1982). However in the absence of outside markers it is not possible to test the model because of the lack of degrees of freedom.

In an attempt to increase the number of recombinant types and therefore to provide the degrees of freedom necessary, some component variables were redefined and the model extended to allow the analysis of chromatids) so that the wider ratio octads could be included when testing the model. *Ascobolus immersus* seems an ideal candidate to test the new model because of the ability genetically to alter the frequency and spectrum of gene

conversion at the locus *wI* coding for ascospore colour (Helmi and Lamb, *Genetics*, *104*, 23, 1983). For nearly all the data tested an acceptable goodness of fit was discovered, suggesting that the mixed (single and dual hybrid chromatid) model of gene conversion was sound.

From these results it is evident that the probability (s) of a certain specific hybrid base pair being repaired to wild type varies with the type of genetic factors (*ccfs*) present. This variation of s appears to be independent of other components showing that some of the simplifications made by previous workers in testing the model were inapplicable. It may be significant in the search for the mechanism of recombination that the value of δ tends to one or zero indicating large differences in frequency of invasion by strands of opposite polarity (5′3′ versus 3′5′) of a double helix.

15. Restorations and substitutions in correction of mispairs in hybrid DNA during recombination

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In most current models, part of the recombination process involves the replacement of part of one strand of a DNA double helix by a corresponding part of one strand from a homologous but non-sister chromatid. Sites of heterozygosity in the heteroduplex give mispaired or unpaired bases. Any enzymic correction of a mispair or non-pair will either be a restoration to the original base pair present on the invaded chromatid, or a substitution, with correction to the base pair of the chromatid from which the invading strand came. Different conversion classes may have different origins, with respect to production from restorations and/or substitutions, depending partly on whether they come from asymmetric or symmetric hybrid DNA, or from double-strand gap repair.

Kitani and Olive (*Genetics*, *62*, 23, 1969), using heteroallelic crosses in *Sordaria fimicola*, found that restrictions equalled or exceeded (often significantly) substitutions, with the amount of excess being allele-specific. Hastings (*Cold Spring Harb. Symp. Quant. Biol. XLIX*, 49, 1984 and other papers) found that restorations and substitutions were equally frequent for alleles *E1/E2* at the *b2* locus of *Ascobolus immersus*, but that in yeast there were more substitutions than restorations for all six alleles studied at the *his*I locus.

Some of the methods used to distinguish restrictions from substitutions are very dependent on the validity of various assumptions. These assumptions will be discussed and relevant data (Zwolinski and Lamb, unpublished) from *Ascobolus* on their validity will be presented. Implications for recombination models will be discussed.

16. Prospect of recombinant DNA technology in human genetics

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The first application of DNA technology in human genetics was in the prenatal diagnosis of α-thalassaemia in 1976, and the following year saw the first biosynthesis of a human protein (somatostatin) and the establishment of the first genetic engineering company to develop recombinant DNA methods for making medically important drugs (Genentech Inc. in the United States).

In 1978 a close linkage between a disease-producing-gene (in this case sickle cell anaemia) and a restriction fragment length polymorphism (RFLP) was demonstrated, and this approach to the detection of carriers of X-linked disorders and the preclinical and prenatal diagnosis of genetic diseases has been widely exploited since.

Four years ago successes in characterizing certain human cancer genes (oncogenes) were published, and finally, and very recently, gene therapy is attracting serious consideration. These various developments auger well for the future of recombinant DNA in human genetics.

17. The molecular pathology of single gene disorders

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Cloning and sequencing of mutant genes from patients with single gene disorders is providing a wealth of information about the molecular pathology of these conditions. So far, most information has been derived from the inherited haemoglobin disorders, particularly thalassaemia. Apart from the obvious point mutations which can give rise to premature stop codons or frameshifts a number of more subtle and interesting lesions have been found. There are several mutations in the conserved regulatory boxed 5′ to the human

globin genes, a whole cluster of point mutations involving intron/exon junctions or within introns and exons which interfere with normal splicing, and another family of mutations which involve the poly A addition site. In addition there are a large family of deletion disorders at least some of which have important relevance to the regulation of the globin gene cluster. Indeed, work carried out over the last few years in the thalassaemia field has probably given us a good idea of the total repertoire of human gene mutations.

As other genes are cloned a similar pattern is emerging. For example the factor VIII genes of patients with haemophilia are showing a remarkable diversity of defects and similar heterogeneity is turning up in most genetic diseases.

Finally, the haemoglobin disorders have provided a new and recently described family of mutations which seem to be involved directly with changes in the developmental pattern of globin gene expression. This group of conditions is providing extremely valuable information about their potential sites of protein/DNA interaction which may be involved in the activation and suppression of genes at different developmental stages.

This talk will review each of these classes of mutations and describe their relevance in understanding gene regulation.

18. Human evolution

A. Wilson

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My talk will deal with the evolutionary insights emerging from molecular comparisons of human beings with one another. The evidence on which I shall centre attention comes from mitochondrial DNA. Every child gets his or her mitochondrial DNA from the mother exclusively. This simple mode of inheritance makes mitochondrial DNA a marvellous genealogical tool. A second notable feature of this molecule is its high rate of evolution. Mutations accumulate unusually fast in this kind of DNA. So, it gives a magnified view of our evolutionary history.

With the aid of restriction enzymes my coworkers have compared the mitochondrial DNAs of many people around the world and built a genealogical tree. Several points of branching in this tree have been associated with the times at which human beings first colonised Australia, New Guinea and the New World. These calibration points agree in implying that the average rate of sequence divergence is between 2 and 4 per cent per million years and that all the lineages in this tree trace back to one woman, who lived 150 to 300 thousand years ago, probably in Africa. Evidence from diverse studies of nuclear DNA agrees with the idea of an African origin for anatomically modern *Homo sapiens.*

Nonmolecular evidence obtained by studying the remains of ancient bones and tools can also be fitted with this idea. The molecular and fossil data together warrant the suggestion that a big-brained population emerged from Africa within the past 200,000 years. The mitochondrial DNA results indicate that there was no mixing of this population with the *Homo erectus* populations represented in Asia by such famous fossils as Java Man and Peking Man seem not to have contributed any mitochondrial DNA lineages to the present-day gene pool of our species.

19. Oncogenes

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The central problem in cancer therapy is the poor selectivity of current systemic agents against the common solid tumours. The demonstration that unique segments of DNA, constant in location and conserved in evolution are involved in growth control, opens new avenues for basic and clinical research. The functions of the products of these genes needs to be elucidated. Examples of growth control functions include homology to growth factors, surface receptors, protein kinases and cell cycle control proteins. From DNA sequence data peptides predicted to be exposed within intact molecules can be constructed and used to produce monoclonal antibodies to oncogene products. Such antibodies have now been successfully used to demonstrate the intracellular localisation of gene products as well as the cell cycle regulatory role of the c-myc protein. By having a battery of antibodies against the different gene products, their direct clinical application for diagnosis and prognosis has become a reality. Immunohistology and flow cytometry permit the geographical and quantitative analysis of function in normal and neoplastic tissues. Furthermore, by purification and biochemical analysis the molecular basis for their action can be elucidated. It is likely that by the end of the decade, new drugs that inhibit oncoprotein function will be available for clinical trial.

20. Molecular characterisation of human chromosome aberrations

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Identification of gross chromosome aberrations is based on an assessment of chromosome length and banding patterns with the light microscope. Defects involving the equivalent of less than 10 million base pairs are not easily resolved and most of these will be missed in screening for human chromosome abnormalities. Two methods hold promise for improving the detection and characterisation of small aberrations. DNA measurement by flow cytometry may sometimes help to resolve duplications and deficiencies between 1 million and 10 million bp. However, family studies are required to distinguish aberrations from normal chromosome variation in the flow karyotype. The development of chromosome specific DNA markers allows the detailed analysis by *in situ* hybridisation and Southern blotting of presumptive chromosome deletions and duplications and the definition of their breakpoints in terms of the chromosome map. The extent by which these methods can improve the resolution of chromosome analysis will be illustrated in a series of structural abnormalities of the human *X* and *Y* chromosomes.

21. Recombinant DNA diagnosis of new mutation diseases—Lesch-Nyham and Duchenne muscular dystrophy

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Genetic lethal diseases were predicted by Haldane to occur frequently on the basis of new mutational events. Two *X*-linked lethal disorders have been studied in our laboratory by molecular genetic methods. For Lesch-Nyhan syndrome the gene structure for hypoxanthine guanine phosphoryltransferase EC 2.4.2.8 was determined in our laboratory. For Duchenne muscular dystrophy, anonymous junctional and deletion molecular probes which flank the Xp2·1 Duchenne muscular dystrophy locus were isolated from numerous laboratories. Southern analysis of Lesch-Nyhan and Duchenne muscular dystrophy males indicate that a significant percentage of cases occur on the basis of *de novo* deletional events (15 per cent Lesch-Nyhan and 8 per cent Duchenne muscular dystrophy). The majority of Lesch-Nyhan males (85 per cent) have no major gene rearrangements and have mRNA of normal molecular weight and size. We have modified the RNAse method of Myers and Maniatis for deletion of point mutations. In 7 of 14 Lesch-Nyhan males studied, a unique RNAse A cleavage site was identified.

In our study of over 60 Lesch-Nyhan families, all mutations thus far identified are unique. The current estimates for the Duchenne muscular dystrophy gene are large (5–10) and its putative transcript and organisation incomplete. Nevertheless, the RFLP linkage study of new mutation families has been successful in the identification of the gametic origin of new mutation in families. Only deletional gametic events at Duchenne muscular dystrophy and Lesch-Nyhan are comparable since the Duchenne muscular dystrophy gene is incompletely characterised.

The carrier and prenatal diagnostic experience of recombinant DNA methods for the disorders of Lesch-Nyhan, Duchenne muscular dystrophy, and cystic fibrosis (Dr A. L. Beaudet from the Institute of Molecular Genetics) will be reviewed. This experience provides guidelines for recombinant DNA application to heritable disease diagnosis and its relative accuracy.

22. Molecular genetics applied to the development of vaccines

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Viral vaccines have traditionally been based upon attenuated or killed strains of the virus. Modern methods of molecular biology enable viral antigens to be produced in microbial or other cells for development as subunit vaccines and, in principle, can also be used to make attenuated strains. Hepatitis B virus antigens made by recombinant DNA methods have been formulated successfully into vaccines and have provided useful diagnostic reagents. Recent results show that internal viral antigens, as well as coat components, have potential for vaccination purposes.

23. Prospects for human gene therapy

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Human gene therapy is defined as the correction of a genetic disease by the insertion of a functional gene into the patient's defective cells. The likely initial disease candidate for this new therapeutic approach is adenosine deaminase (ADA) deficiency, a cause of severe combined immunodeficiency (a profound genetic disorder which often results in death before the age of two). Progress towards gene therapy is proceeding along two pathways: scientific and sociolethical. A number of laboratories have been able to use retroviral vectors to insert a functional gene into the hematopoietic cells of mice. We have now succeeded in transferring the human ADA cDNA into the hematopoietic cells of nonhuman primates using an autologous bone marrow transplantation/gene transfer protocol. In the most positive monkey, approximately 1 cell in 200 of the hematopoietic mononuclear cells appears to be carrying and expressing the human ADA gene. In addition, we have demonstrated the feasibility of gene transfer *in utero* using fetal lambs. On the socioethical front, mechanisms are now in place for evaluating in a public forum human gene therapy proposals. In addition, Points to Consider (*i.e.*, "guidelines") have been published by the U.S. Government to inform investigators what scientific and ethical criteria need to have been met at the time a clinical protocol is submitted.

24.

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25. Human chromosome 19 and the myotonic dystrophy gene

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Human chromosome 19 is currently the most fully mapped of the smaller chromosomes, with over 40 loci assigned to it. cDNAs are available for genes and at least 10 random DNA segments have been cloned. Four genes encoding cell surface antigens of unknown function, identified by monoclonal antibodies also map to chromosome 19.

We have assembled a panel of rodent human somatic cell hybrids which provide a comprehensive breakdown of this chromosome. These lines have been used to order many of the markers and along with multipoint linkage analysis to establish the position of the myotonic dystrophy (DM) gene. Some of available methods for isolating the DM gene, given its precise localisation relative to other genes, will be discussed.

26. Karyotyping of various yeasts by field inversion gel electrophoresis

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We have shown by use of OFAGE that many laboratory and industrial strains of *Saccharomyces* (spp. *cerevisiae, uvarum, carlbergenesis, bayanus*) produce generally similar patterns of chromosomal DNA (12–17 bands, the majority of which are 100 kb approximately) (Johnston & Mortimer, *Int. J. Syst. Bact.*, *36*, 569, 1986). However, because of chromosome polymorphism, most of these strains can be identified by specific OFAGE karyotypes. We have also shown that strains of *Saccharomyces kluyveri, Candida albicans, Candida utilis, Kluyveromyces lactis, Pichia (Hansenula) canadensis, Saccharomycopsis fibuligera* and *Schwanniomyces occidentalis* produce only a few slowly-migrating bands, indicating that these yeasts possess a smaller number of larger chromosomes (1000 kb). We speculate that "domesticated" species of *Saccharomyces* may have evolved from a progenitor wild species, such as *S. kluyveri*, by selection for sugar fermentation genes, e.g. MAL, SUC, and their consequent transposition along with telomeric sequences. Similar results for a wide range of yeasts have recently been obtained by Jonge *et al.* (*Yeast*, *2*, 193, 1986).

The demonstration that, under certain conditions, the largest chromosomes of *S. cerevisiae* migrate at a faster rate than medium-sized chromosomes during FIGE (Carle, Frank and Olson, *Science*, *232*, 65, 1986) suggests an improved method for examination of the large chromosomes of *S. kluyveri* and other yeasts. We have shown this to be the case and obtained greater resolution

of chromosomal bands of these yeasts. Our current estimates are that most of these yeasts probably possess 4–6 chromosomes, and *Saccharomycopsis* probably only 2 or 3. We have also shown that the 3 large chromosomes of *Schizosaccharomyces pombe*, estimated to contain between 3 to 6 Mb DNA, migrate at faster rates than chromosomes estimated at 2–2·5 Mb under particular FIGE conditions. Resolution of the 3 chromosomal bands of *S. pombe* has been obtained with shorter run times of 10–20 hours.

We have probed Southern blots of FIGE gels and identified chromosomes III (*LEU2*), IV (*TRP1*), V (*URA3*, *RAD24*), VI (*TUB2*) and possibly XII (*SIR3*) of *S. cerevisiae*. Under conditions of high stringency, no hybridisation was observed between *TRP1*, *RAD24*, *URA3*, *TUB2*, and chromosomal bands of several other yeasts. However, with reduced stringency, homology with the *S. cerevisiae* probe pRB129 carrying *URA3* and *TUB3* has been observed, generally involving 2 FIGE bands of these yeasts.

The resolution of these larger chromosomes suggests the application of FIGE to karyotyping other eukaryotic microbes, such as filamentous fungi and protozoa and to separating large DNA fragements of chromosomes of higher eukaryotes.

27. The expression of chromosome 21 specific sequences in normal and Down Syndrome cell lines and tissues

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Human DNA isolated from a genomic library enriched for chromosome 21 sequences was digested with Sau3A, subcloned into pUC12 and used to transform *E. coli* JM83 cells. On screening the resulting transformants with a trisomy 21 foetal liver cDNA probe, several positively hybridising recombinants were detected. A comparison of the expression of 6 of these human chromosome 21 DNA sequences in normal and Down Syndrome fibroblast and foetal liver samples and performed by dot blot analyses followed by densitometer studies. The results of these comparisons have revealed one sequence which is expressed in Down Syndrome foetal liver but not in normal foetal liver at an equivalent stage development, one sequence which is expressed more than ten times as much in Down Syndrome tissues and cell lines as in normal tissues and cell lines and one sequence which shows no difference in expression levels in the trisomy state. Other sequences show a 3 : 2 ratio of expression between the Down Syndrome and the normal samples. These results suggest that the multiple phenotypic effects observed in trisomy 21 subjects may be due to changes in the developmental regulation of transcription, loss of tissue-specific gene expression and widely varying dosage effects of different DNA sequences. Although it is widely believed that the phenotypic expression of Down Syndrome is due to a 1·5-fold increase in the expression of most, if not all, chromosome 21 specific sequences, this study indicates the need to examine the expression of individual sequences in different tissues prior to considering their role in the pathogenesis of Down Syndrome.