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(Abstracts of papers presented at the TWO HUNDRED AND SEVENTH MEETING of the Society held from 13th to 14th November 1987 at UNIVERSITY COLLEGE, LONDON)

1. Molecular cloning of the chromosomal breakpoint associated with increased tumorigenicity in a HRAS1-selected chromosome-mediated gene transformant

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Introduction of mitotic chromosomes from the human EJ bladder carcinoma cell line into mouse C127 cells, with selection for cellular transformation, generated a series of transformants all of which carry and express the HRAS1 oncogene on varying lengths of human chromatin.

One transfectant, E65·5, shows a 100 fold increased level of tumorigenicity over the other transformants but with no detectable change in expression of the HRAS1 gene.

Genomic restriction analysis shows that E65·5 is exceptional in that although all restriction sites 5′ to and within the coding regions are retained, all sites that lie just 3′ to the HRAS1 locus have been lost. A variable repeat region (vtr) probe shows no detectable hybridisation.

As an initial step towards understanding the nature and possible mechanism of the chromosome translocation, particularly with a view to the raised tumorigenicity, the rearranged HRAS1-hybridising fragment has been isolated from a size-selected genomic library cloned into the phage vector EMBL3. Fine restriction mapping of a sub-cloned fragment establishes that the coding sequences are intact and that the complete vtr has indeed been lost, the break point lying within 700 bp from the HRAS1 polyA addition site.

2. DNA methylation and genetic instability in tumour cells

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There is increasing evidence that many of the changes in phenotype observed in tumour cells are the result of epigenetic rather than genetic events. Much of this may be due to alterations in the pattern of DNA methylation. We have shown that in the Friend erythroleukaemia cell line, which has a high mutation rate at the thymidine kinase (TK) locus, treatment of TK-mutants with 5-azacytidine caused reversion in up to 32 per cent (Hickey *et al.*, *Exp. Cell Res.*, *164*, 251, 1986). Cells in which the TK gene has been reactivated in this way can become very unstable and show high frequency switching between TK^+ and TK^-. Non-mutant TK^+ cells show an elevation of approximately 50 per cent in thymidine kinase activity after treatment with 5-azacytidine and this is maintained for at least 200 cell generations in culture. However some clones also show frequencies of TK-mutants between 10^{-4} and 10^{-3} suggesting that aberrant re-methylation may have occurred in some cases.

In lymphoma P388 cells, another cell line with high mutation rate at the TK locus, 5-azacytidine treatment also increased the frequency of TK^+ revertants, but here the response was much more limited.

Systems such as the Friend erythroleukaemia TK locus where an already unstable gene can be made hyper-unstable should provide useful models in which to study the factors responsible for tumour cell heterogeneity.

3. Pleiotropic effect of scale pattern genes in common carp: susceptibility to *Ichthyophthirius multifiliis* infection

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Scale patterning in *Cyprinus carpio* (common carp) is controlled by two pairs of autosomal alleles. The pleiotropic effect of these genes has already been established for a number of characteristics (Kirpichnikov, *Genetic Bases of Fish Selection*, Springer-Verlag, 1981). We have examined the possible effect on resistance to *Ichthyophthirius multifiliis* infections. *I. multifiliis* is a ciliated protozoan and a common ectoparasite of freshwater fish throughout the world, causing the disease known as 'Ich' or white-spot.

All four scale pattern phenotypes of carp were compared at three different temperatures and using two isolates of parasite. Fish were exposed to standardized doses of *I. multifiliis*, and response to infection was standardized to the fish's body area. Variation in susceptibility to infection was observed, as was variation in infectivity of the two parasite isolates.

Our observations indicate a level of genetic variation which is worth further examination. Selection of resistant phenotypes may possibly be effective in producing resistant strains of fish for use in aquaculture. We intend to pursue this work in the future.

4. Utilisation of reverse-phase high-performance liquid chromatography in screening for polymorphism among strains of *Coprinus cinereus*

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Cytoplasmic ribosomal proteins prepared from five related haploid strains and two diploid strains of *Coprinus cinereus* were analysed by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). The 80S-ribosomal proteins from these strains were resolved into approximately 35 protein peaks using μ-Bodapak C_{18} column and a trifluoroacetic acid/acetonitrile solvent system. Although the RP-HPLC elution profiles for the haploid strains were essentially similar, there were consistent differences in the protein peaks between the strains, indicating that polymorphism in this organism extends to the cytoplasmic ribosomes. The RP-HPLC profiles for the diploid strains showed the presence of proteins from both haploid parents.

The results presented illustrate the effectiveness of the RP-HPLC method in screening for polymorphism among strains of *Coprinus cinereus* at the level of ribosomal proteins. Compared with the traditional methods RP-HPLC is easier to perform, more rapid and precise, and generally affords better reproducibility.

5. Gene transfer between basidiomycete and ascomycete fungi by cotransformation

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Contransformation provides a technique for introducing an unselected gene into a suitable host without the need to make complex plasmid constructs with more than one gene. We have used cotransformation to ask whether genes from ascomycete and basidiomycete fungi can be interchanged. Three genes from the ascomycete *Aspergillus nidulans* (*trpC*, PRA isomerase; *acuD*, isocitrate lyase and *facA*, acetyl CoA synthetase) were introduced into the basidiomycete *Coprinus cinereus* and tested for ability to complement mutations in the analogous genes (*trp*-2, *acu*-7 and *acu*-1). The *trp*-1 (tryptophan synthetase) gene was used as the selectable marker and some 100 *trp*-1^+ transformants from each experiment were tested for expression of the *A. nidulans* gene. None of the ascomycete genes were expressed in any of the transformants tested. In a similar experiment, the *trp*-1 gene of *C. cinereus* was introduced into *A. nidulans* and shown not to be expressed. Southern blot analyses confirmed that at least 30 per cent of the transformants isolated contained at least one copy and generally several copies of the unselected heterologous gene. Control experiments using a *trp*-1 *acu*-7 mutant of *C. cinereus* showed that up to 60 per cent of the selected *trp*-1 transformants could be expected to express the

unselected gene. We conclude the lack of expression of the heterologous gene was due to essential differences in promoter sequences rather than due to inactivation during integration.

6. Fractionation of active messenger RNA from *Coprinus cinereus* on sucrose density gradients and oligo (dT)8 trisacryl-M columns

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RNA was extracted from *Coprinus cinereus* using guanidium thiocyanate followed by centrifugation through caesium chloride. Fractionation on sucrose density gradients indicated that most of the active mRNA did not bind oligo (dT)8 Trisacryl M columns; about 10 per cent of the mRNA bound to the column and eluted in two separate fractions of differing activity in translation assays.

Proteins produced by *in vitro* translation of fractionated mRNA were examined by fluorography after electrophoresis on Laemmli gradient gels.

7. *In vitro* fertilization of hamster oocytes by bovine spermatozoa

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The use of zona-free hamster oocytes in the sperm penetration assay for the assessment of sperm fertility and the visualization of sperm chromosomes has only recently been extended to bovine spermatozoa. This communication reports on the use of dilauryl phosphatidylcholine liposomes (PC12) to rapidly induce an acrosome reaction in frozen-thawed bovine spermatozoa leading to penetration of zona-free hamster oocytes (Graham *et al.*, *Biol. Reprod.*, *35*, 2, 413–424, 1986) and their subsequent culture for the visualization of bovine sperm chromosomes.

Concentrations of PC12 that induce the acrosome reaction are correlated with the penetration of zona-free hamster oocytes reaching maximum penetration at 50 μM PC12. The efficiency of production of sperm chromosome spreads depends upon penetration rates of zona-free oocytes and decondensation of the sperm heads within the ooplasm. Following penetration, the majority of spermatozoa failed to fully decondense or form male pronuclei. Therefore, treatment with PC12 liposomes is a suitable regime for acrosome reacting bovine spermatozoa for the sperm penetration assay but not for the sperm chromosome assay as the efficiency of bovine sperm chromosome production is low.

8. The application of multiple banding techniques, alone and in conjunction with image analysis, to the study of human sperm chromosomes

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Q-banding has remained the pre-eminent means of cytogenetically analysing human sperm chromosomes since they were first demonstrated (Rudak *et al.*, *Nature*, *274*, 911, 1978) by heterospecific *in vitro* fertilization of zona-free hamster eggs. However, we can confirm the observations of Kamiguchi and Mikamo (*Am. J. Hum. Genet.*, *38*, 724, 1986) that attaining well resolved Q-bands in sperm chromosomes is not a reliable process. While this phenomenon may be connected with post-fertilization culture conditions, the additional diagnostic capability that would be derived from the choice of applying some of the binding techniques routinely used in other cell systems, would be most welcome. Sele *et al.* (*Ann. de Genet.*, *28*, 81, 1985) have demonstrated selective denaturation R-bands and Benet *et al.* G-bands after combined 2X SSC and trypsin treatment (*Hum. Genet.*, *73*, 181, 1986). This latter method would seem to be a minor derivation of a more general technique described by Burgos *et al.* (*Strain Tech.*, *61*, 257, 1986). We have obtained consistently good fluorescent R-banding using chromomycin-A3 and methyl green counterstain. More recently, we can confirm that counter staining can be dispensed with if the *p*H of the mountant medium is raised.

G-bands have been attained by a combination of pre- and post-fixation methods. The former approach rather elegantly circumvents many of the temporal demands of banding, provided the majority of eggs are fertilized synchronously with final

visualization by silver staining. In cases where final chromosome quality is sub-optimal, we have investigated the value of applying image analysis enhancing routines. Implementation of algorithms that specifically detect band break-points have been found most useful.

9. Allosuppressor mutations isolated in *Aspergillus nidulans* as antagonists of antisuppressor mutations

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Allosuppressor mutations have been isolated following UV mutagenesis of a strain containing *suaC*109, *asuB*11 or *asuD*14 and *alX*4. *suaC*109 is a ribosomal suppressor which permits an *alX*4 strain to grow on allantoin as nitrogen source. *asuB*11 and *asuD*14 are antisuppressors of *suaC*109 (Martinelli, *Genet. Res.*, *49*, 191–200, 1987), and also partially reverse the cold-sensitive, drug-sensitive phenotype of *suaC*109. Allosuppressor strains grow better on allontoin than one parent strain (*alX*4, *suaC*109, *asu*). These new mutations also variously alter the pleiotropic phenotype with respect to sensitivity to cold, cycloheximide and hygromycin. They could therefore be mutations in genes coding for ribosomal components.

All the allosuppressor mutants have been crossed to wild type strains. The *alo*˜ mutations are all extragenic to both *suaC* and the *asu* genes. Progeny from these crosses have been back crossed to parental and control strains in order to match the few phenotypic classes to genotypes. It is suspected that the $alo^{\sim}sua^{+}asu^{+}$ combination is lethal and that $asu^{\sim}alo^{\sim}sua^{+}$ is normally the same as $asu^{+}alo^{+}sua^{+}$. In some cases $alo^{\sim}sua^{\sim}asu^{+}$ progeny have been identified. This could indicate that the allosuppressors act by enhancing suppressor *suaC*109 rather than by reducing the antisuppressor activity.

10. Mutagenicity studies on tobacco smoke condensates

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The effects of three cigarette smoke condensates on mutation frequencies at the thymidine kinase (TK) and the hypoxanthine guanine phosphoribosyl transferase (HGPRT) loci in L5178Y ($TK^{+/-}$) mouse lymphoma cells were investigated. An exposure time of 4 hours was used. Two of the cigarette tars were derived from middle tar cigarettes, while the third tar was derived from middle to high tar cigarettes.

In the absence of S9 mix the middle to high tar smoke condensate induced a weak but dose-dependent increase in mutation frequency at the TK locus and a weak but dose-independent increase in mutation frequency at the HGPRT locus. The other two condensates showed no mutagenic activity at either locus in the absence of S9 mix.

In the presence of aroclor-induced S9 mix, none of the condensates showed mutagenic activity at the TK locus. Both of the middle tar smoke condensates showed dose-dependent increases in mutation frequency at the HGPRT locus. The middle to high tar smoke condensate showed no mutagenic activity at the HGPRT locus in the presence of S9 mix.

11. Genetic fingerprinting of birds of prey

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Genetic fingerprinting is a new and very powerful technique for the unique identification of individuals and their familial relationships.

Examples will be given of its application to population genetics and conservation of birds of prey.

12. DNA fingerprinting of house sparrows

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The use of DNA fingerprinting in the determination of relatedness between individual birds is

demonstrated in several families of house sparrows. The technique allows parentage to be proved unequivocally and has enabled us to identify the true parents of "illegitimate" offspring.

13. Chairman

G. Bulfield

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14. The bovine aspartyl protease chymosin is a member of a multigene family

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Chymosin is a developmentally regulated aspartyl protease produced by the 4th stomach of the neonatal calf for a few weeks after birth. It catalyses the cleavage of a single peptide bond in kappa-casein. It is related to pepsin, renin, cathepsin D and cathepsin E. A complete chymosin cDNA has been cloned and sequenced. It is different from 8 other chymosin DNA sequences. Surprisingly all nine are different from each other. Many of the differences lie in *E. coli dam* methylase sites (CC A/T GG) suggesting Maxam and Gilbert sequencing errors. Excluding the differences at *dam* sites the 9 chymosin DNA sequences encode 6 different proteins which differ on average by 1-2 amino acids. Restriction maps of 10 genomic clones (each 10-20 kb), detected under stringent conditions by hybridisation to chymosin cDNA, show, contrary to previous reports, that there are multiple sequences closely related to chymosin cDNA. If these encode chymosin the high degree of polymorphism would be partially explained. If they encode proteins closely related to chymosin it will be interesting to discover if they are aspartyl proteases with different biological specificities than chymosin.

15. Gene conversion control factors and their evolutionary effects

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Conversion control factors (*ccfs*) are widespread in fungi, occurring *e.g.* in Ascobolus, Coprinus, Neurospora, Schizophyllum, Schizosaccharomyces and Sordaria. In Pasadena strains of *Ascobolus immersus*, derived from just two haploid ascospores, a survey of 40 loci showed clear evidence of different *ccf* polymorphisms affecting 16 target loci, no evidence of *ccf* polymorphisms affecting 5 loci, and inconclusive results for 19 loci.

There are three major types of *ccf*. "Heterozygous reducers", such as *ccfs* 1, 5, 6, *cv*, and *ss*, are closely linked to target loci and reduce the latters' conversion when the *ccfs* are heterozygous. "Closely linked effectors" which are not heterozygous reducers, such as *ccf*2, *cog*, M26, YS17, affect conversion of closely-linked target loci; they may show *cis*/*trans* effects and may act as receptors for control by "effectors not closely linked", such as *ccfs* 3 and 4, *rec*-2, *RecE*, *RecR*, which act over long distances, often being unlinked to their target locus.

Experimental studies of the effects of *ccfs* on conversion of target loci have been combined with theoretical studies and simulations to examine the effects of *ccfs* on the population genetics of alleles at target loci. Polymorphism for "heterozygous reducer" *ccfs* slows down conversion-directed changes in allele frequencies at target loci. For "closely linked effectors" and "effectors not closely linked", the particular *ccf* alleles present in a population can affect the direction and rate of conversion-directed changes in target locus allele frequencies, and equilibria, in complex ways. Linkage disequilibrium between target loci and closely linked *ccfs* will usually be very persistent, reducing by different amounts for different *ccf* allele/target locus allele combinations.

16. Characterisation of the murine homeo-box-containing gene Hox-1·6

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Hox-1·6 is a murine homeo-box-containing gene that shows more divergence from the *Drosophila*

Antennapedia-like homeo-box class than other members of the Hox-1 locus (A. Baron *et al.*, *EMBO J*, in press 1987). It is expressed during embryogenesis and in an intestine-specific manner in adults, as well as in endodermal-like tumours and cell types. The sequence of Hox-1·6 embryonic cDNA clones and their use in S1 analysis revealed an alternative splicing pattern for Hox-1·6 transcripts. We propose that these differentially spliced molecules are translated to yield a set of related proteins.

17. Chairman

A. Radford

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18. The prevention of genetic disease: the case for more human genetics in the school curriculum

J. Fitzsimmons

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19. Genetic concepts and the ability of children to understand them

E. Little

Department of Education and Science.

20. Issues in genetic education of social importance: race, intelligence and mental illness

J. A. Stewart

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21. Genetic topics other than human/medical which are relevant to basic science education

D. J. Cove

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22. GCSE Biology and its genetic content: the syllabus and available texts

M. Tribe

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23. Chairman

A. McLaren

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24. Methylation and gene expression during early mouse development

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Global DNA methylation was investigated as a possible molecular basis for X-chromosome inactivation and reactivation and the memory or imprint distinguishing maternal and paternal genomes. The egg genome is undermethylated and the sperm genome relatively methylated. There is a striking loss of global methylation during preimplantation development then *de novo* methylation begins about the time of implantation, first in the foetal precursor cell DNA and later, and to a lesser final extent, in extraembryonic cell DNA. *De novo* methylation is slow, occurring over several cell cycles. Independent methylation in space and time in different lineages may be correlated with differential programming. Foetal germ cell DNA is markedly undermethylated and this may be a precondition for X-reactivation, erasure of imprinting and reprogramming of the germ line to developmental totipotency.

25. Temporal and tissue-specific expression of two Ca^{++}-binding extracellular proteins—SPARC (osteonectin) and 2ar (osteopontin) during mouse development

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Differential screening of a cDNA library from parietal endoderm—an extraembryonic tissue specialised for the production of extracellular matrix—led to the isolation of a cDNA for SPARC (a secreted protein which is acidic and rich in cysteine) (Mason *et al.*, *EMBO J. 5*, 1465–1472; 1831–1837 1987). Mouse SPARC has subsequently been shown to be highly homologous to bovine osteonectin, a CC^{++}-binding protein present at high levels in bone and dentine. By Northern analysis and *in situ* hybridization we have shown that SPARC is expressed by a wide variety of cell types in embryonic and adult tissues, and is not confined to mineralised-matrix producing cells. Expression of SPARC increases approximately twenty fold in F9 teratocarcinoma stem cells treated with retinoic acid and cyclic AMP. This is due in part to an increase in gene transcription, and recent results concerning the DNA sequences responsible for regulating this expression will be presented.

2ar is also a C^{++}-binding glycoprotein, and was originally isolated as a gene induced in cultured mouse epithelial and fibroblast cells by tumour promoters (Smith and Denhardt, *J. Cell. Biochem.*, in press 1987) and growth factors such as epidermal growth factor, basic fibroblast growth factor and embryonal carcinoma derived growth factor. Sequence analysis has shown that mouse 2ar is identical to rat osteopontin, an RGD-containing protein present at high levels in bone. We have used *in situ* hybridization to study the localisation of 2ar transcripts during mouse bone development, and to compare this with SPARC expression. The results suggest that the two genes are expressed by different populations of bone-associated cells. 2ar is also expressed at high levels by granulated metrial gland cells in the deciduum and placenta. These are maternal bone-marrow derived cells, the function of which is at present unknown. 2ar expression is also seen in the medulla of the kidney and in a few other cell types. Possible functions of SPARC (osteonectin), 2ar (osteopontin) and of granulated metrial gland cells will be discussed.

26. Developmental expression of murine homeo box and Kruppel homologue genes

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It is known for Drosophila that DNA-binding proteins are responsible for the regulation of a number of vital developmental control processes, as exemplified by homeotic gene products. The genetic tools which facilitate the study of Drosophila cannot be applied readily to the more complex mammalian genome. One way of overcoming this disadvantage is to isolate related genes by identifying homologous sequences. Thus the Drosophila homeo box applied as a probe enabled us to characterize a family of mouse genes on chromosomes 6, 11, and 15 which contain homeo boxes. We then studied the expression of these genes during development, *in vitro* in embryonal carcinoma cells, using F9 and p19 cells as models, and *in vivo* during embryogenesis, using the mouse as a model. Our results showed that Hox-1·1 is temporally regulated in F9 and p19 cells and, in spite of the fact that stable RNA could not be found in F9 stem cells, the Hox-1·1 gene was seen to be expressed transiently after differentiation of the cells into parietal endoderm. Characteristics of the protein used to produce antibodies confirming the transient expression of Hox-1·1 are a length of 229 amino acids and molecular weight of 31 kD in SDS gels. Moreover, its location is in the nucleus and it has been found in association with chromatin. Our *in situ* analyses revealed the expression of these genes to be restricted spatially depending on the gene analyzed. Specifically, the Hox-1·3 gene is spatially and temporally restricted in its expression to murine embryonic structures originating from or induced by the mesoderm. In particular, expression seems to be spatially limited to the thoracic region, especially to components

of segmental origin, such as embryonal ribs and vertebrae, and their precursors, such as the equivalent sclerotomes, somites, and presomitic mesoderm. In addition, expression can be found in parts of embryonal lung, stomach tissue, gut, and kidney, tissues whose formation is based on induction of region-specific mesoderm. Interestingly, while Hox-1·3 is expressed in somites 8–22, Hox-1·1 is expressed in somites 10–16. This could point to an involvement of these genes in the formation of these individual structures. In the same way we used the homeo box to look for conserved DNA-binding domains, Krüppel also enabled us to find a second family of mammalian genes defined by their potential "finger" structure. The sequences of two separate isolates were analyzed, revealing seven possible "fingers" with a putative metal-binding domain. By using these two genes as probes their expression could be monitored during F9 cell differentiation, where it was found that they are active, unlike homeo box genes (*e.g.*, Hox 1·1). Both genes are "switched off" by differentiation of the cells into parietal endoderm. We are concentrating our present studies on defining what role these genes play in mouse development.

27. Attempts to do inverse genetics in the mouse

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The advent of direct isolation into tissue culture of pluripotential cells from the early mouse embryo (EK cells) has allowed the route from tissue culture to the whole animal and its germ line first envisaged for teratocarcinoma stem cells to be fully realised. EK cells may be readily isolated from explanted mouse blastocysts and maintained in tissue culture. Following genetic transformation selection and cloning these cells are able to recolonise a murine embryo and participate in the formation of germ line chimaeric mice.

The most important practical differences between this route to transgenesis and that of zygote injection is that the time period in vitro may be used both to allow efficient multiple cell transformation and to allow selection of specific rare events.

Screening for insertional mutation both *in vitro* and *in vivo* will be discussed.

28. Transcription of the mouse $\alpha 1$(I) gene carrying the Mov-13 insertion

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In the Mov-13 mouse, an embryonic recessive lethal, insertion of a Moloney leukemia provirus into the first intron of the gene for the $\alpha 1$ chain of collagen I has resulted in a complete transcriptional block in all cells of 11-day embryos or (fibroblastic) cell lines derived from them. Consequently, homozygous embryos cannot synthesize collagen I. In contrast, normal teeth with massive dentin layers (composed mainly of Collagen I) are formed in tooth transplants derived from 13·5 d rudiments of homozygous Mov-13 embryos. *In situ* hybridization experiments indicate active transcription of the mutated $\alpha 1$(I) gene in odontoblasts and show the presence of the cellular transcript running (in opposite direction) through the Moloney insert. Presumably, this viral (antisense) sequence is subsequently removed with the first intron through normal mRNA processing. The dramatic difference in the effect of the provirus insertion on transcription in fibroblasts and in odontoblasts reveals different modes of transcriptional regulation of the same gene in two cell types.

29. The expression of actin and myosin genes during striated muscle development in the mouse

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During the formation and maturation of striated muscle, different isoforms of the actin and myosin multigene families are expressed. Thus in skeletal muscle, during the later stages of foetal development in the mouse, there is co-expression of the adult cardiac and skeletal actin and myosin light chain isoforms, while after birth only the skeletal isoform accumulates. In contrast, for the myosin heavy chain family, there is sequential expression of specific developmental isoforms in foetal/neonatal skeletal muscle. These genes are linked in a

single gene cluster on mouse chromosome 11, whereas the actin and myosin light chain genes are dispersed.

In situ hybridization with specific actin and myosin gene probes is a technique which makes it possible to look at the expression of these gene families at much earlier stages of striated muscle formation and in individual cells and fibres. Thus cardiac and skeletal actins are observed to have distinct spatial and temporal distributions in the somites of the mouse embryo. The presence of different precursor muscle cell populations, giving rise to different types of muscle fibres can be approached in this way. At later stages of foetal development primary and secondary fibres have been described; formation of the latter is dependent on innervation. The developmental transitions in myosin heavy chain gene expression are observed in β-bungarotoxin treated embryos and therefore a) are independent of innervation and b) can take place in the primary fibre population alone.

Certain inbred mouse lines (*e.g.*, BALB/c, DBA/2) contain a mutated cardiac actin locus which results in lower levels of this gene transcript and protein. There is a compensatory increase in skeletal actin in this situation, suggesting regulatory coupling of cardiac and skeletal actin genes. This mutation makes it possible to examine how these genes interact with each other during striated muscle development.

30. Hypervariable DNA and genetic fingerprints

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