Conference report

Symposium on genomic imprinting, Manchester

A symposium on Genomic Imprinting was held in Manchester on 4 to 6 April 1990 as part of the 1990 Joint Meeting of the British Society for Developmental Biology, the British Society for Cell Biology, and the Anatomical Society of Great Britain and Ireland. This symposium was organised by Drs Marilyn Monk and Azim Surani, and consisted of five sessions; of these, four sessions were devoted to imprinting in animals and one was devoted to plants. I greatly enjoyed the symposium because of the challenge posed to us students of human genetic disease by advances in the understanding of the genetics of other organisms. In addition, the meeting had a refreshing informality that stimulated useful discussions.

Imprinting can be defined as the mechanism whereby the activity of homologous genes in a person will differ, depending upon the sex of the parent of origin. Uzi Nur reviewed the phenomenon of heterochromatinisation in certain insects, in which chromosomes of paternal origin may be rendered heterochromatic at the discretion of the female parent. This process is under direct genetic control and is intimately related to sex determination, so these female insects can adjust the sex ratio of their offspring. Tartof reviewed studies of position effect variegation in *Drosophila*. I learned of the existence of trans acting, dominant genes that enhance or suppress the expression of the mutant phenotype caused by the inappropriate juxtaposition of heterochromatin and the target gene. Several such modifier loci exist: enhancers and suppressors are often allelic and may represent duplications or deletions of the same sequences, which perhaps code for the protein components of heterochromatin.

Sharat Chandra related the evolution of imprinting to the evolution of sexual dimorphism. In certain insects, imprinting of an entire set of chromosomes is the mechanism of sex determination. Imprinting could assist gamete differentiation in organisms acquiring the habit of anisogamy, but would be unlikely to confer a direct advantage otherwise, through the suppression of heterosis. Could imprinting result in a memory in each haploid genome of its history over several generations, or would there have to be an erasure effect at each passage through a gonad? (Answers on a postcard to . . .) Alternatively, imprinting may have arisen because it co-evolved with other traits, or the genes responsible for imprinting may have advantageous, pleiotropic effects.

Marilyn Monk reviewed mammalian X chromosome inactivation, the possible mechanisms involved, and the evidence pointing to genetic control of the process. She explained that methylation studies on the X chromosome bear no simple relationship to gene activity or chromosomal replication. Whereas cytosine residues 5' to a gene may be methylated on the inactivated X, the reverse may apply to cytosines within the body of the gene. There may well be critical sites, for methylation or some other modification process or both, which are always correlated with gene activity. Patterns of methylation differ greatly between tissues at different stages of development: sensitive, quantitative PCR analysis of X chromosome sequences is being developed to examine methylation differences.

Peter Jones described experiments that used 5' azacytidine to inhibit the maintenance methylase of undifferentiated, uniformly methylated cells: this enzyme methylates the unmethylated strand in the hemimethylated molecules resulting from chromosome replication. These cells were induced to differentiate, stably, along several different paths: those that became myogenic cells were found to produce a factor that could induce other cells to differentiate the same way. The MyoD gene produces this factor: its pattern of methylation varies between tissues, but in no very clear fashion. Jones also discussed the possible relationship between differential rates of mutation and of methylation: methylation of a cytosine residue may predispose to deamination to thymidine, as happens in the p53 gene on 17p.

Bruce Cattanach reviewed the experiments that showed the marked phenotypic consequences of uniparental disomy for some regions of the mouse genome. These studies have used reciprocal crosses between translocation carrying mice to achieve uniparental disomy (upd) for not only whole chromosomes, but also for chromosomal regions. Interestingly, the phenotypic changes caused by a given upd will vary in opposite directions from those caused by the opposite upd. Then, Solter's work with pronuclei and with transgenes was reviewed by DeLoia. One transgene inserted into mouse chromosome 5 has resulted in defective digit formation, but only in the offspring who inherited the defect from their male parent.

Charles Babinet described an ingenious series of experiments in which he manipulated male and

female pronuclei and cytoplasm. He and his team found no evidence that imprinting influenced gene expression before the eight cell stage of mouse development, but also studied a strain of mouse (DDK) in which the females are effectively sterile when crossed to other strains: early embryonic lethality is the rule in this situation, but not in reciprocal crosses. DDK cytoplasm interacts with a non-DDK paternal genome to prevent the normal development of blastocysts: differential expression from the parental genomes must have occurred. Azim Surani presented results of work with two different transgenes in mice. In one case, expression varied with the mouse genetic background and with parental origin, but there was no correlation with methylation of the transgene. Another example did display a correlation of transgene activity with methylation. In this case, there were cumulative changes in DNA methylation acquired over several generations, and back crossing to inbred lines showed the effects of modifier genes. Could this relate to the genetic anticipation seen in myotonic dystrophy, for example?

Wolf Reik described his results in two studies of gene methylation. In the mouse *Sparc* gene, the methylation of sites at exons 9 and 10 seems to be sex independent until backcrosses to two different parental strains are performed. These crosses show that the gene methylation pattern differs depending upon the sex and strain of the parent of origin. Other studies of the human 4p region have shown non-Mendelian inheritance of methylation patterns revealed by a marker close to the Huntington's disease locus. If modifier genes are involved in sex or strain specific methylation effects, as is likely, then surely these genes must modify more than single CpG sites? Carmen Sapienza briefly reviewed the evidence of imprinting found in yeasts, *Chlamydomonas*, maize, and various invertebrates. He then discussed the possible role of imprinting in oncogenesis: perhaps imprinting could act as the first of Knudson's two hits? He also described the variegated phenotype displayed by many traits whose controlling genes are liable to imprinting (and not just those on the X chromosome). This might indicate the effect of modifier genes that influence imprinting at other loci. Any X chromosome gene that influenced methylation could result in imprinting, because of the possibility of parental sex effects.

Only on the final day was *H sapiens* the prime focus. I reviewed some of the evidence that imprinting is a human phenomenon. At the level of the genome, there is the androgenetic origin of hydatidiform moles, the gynaegenetic origin of ovarian teratomata, and the differences between diandric and digynic triploid conceptions. At the level of the single locus, Huntington's disease (HD), myotonic dystrophy, and some family cancer syndromes have been

suggested as examples of imprinting, with varying degrees of justification. I also discussed Charles Laird's intriguing models of imprinting in relation to HD and to the fragile X mental retardation syndrome. Judith Hall proceeded to discuss the evidence that imprinting may be found in humans as a phenomenon at the level of the chromosome. Mouse homologues were considered to indicate areas of the human genome that might be subject to imprinting, including human proximal 15q, in relation to Prader-Willi and Angelman syndromes. The role of chromosome loss from trisomic conceptions was considered both as an aid to fetal survival when the placenta ejects the additional chromosome, and as a possible cause of uniparental disomy. She also considered the pedigree structures to be expected for disorders in which imprinting may be of clinical importance, and speculated as to the possible role of imprinting in relation to monozygotic twinning. A nomenclature to be used by clinicians in discussing uniparental disomy was proposed. She finally discussed the possibility that cross talk between homologous chromosomes may occur during early meiosis, when telomeres are still embedded in the nuclear membrane, and that this may be of great importance in imprinting. The work of Maj Hultén in studying the behaviour of human chromosomes at male meiosis was acknowledged.

The final contribution to the symposium was that of Robin Holliday. He discussed allelic exclusion, as an all or nothing phenomenon (other forms of imprinting may modulate gene activity rather than switch genes on or off). He outlined experiments with cell lines in vitro, in which recessive phenotypes appeared as a functional hemizygosity, with a single defective allele. Reversion to the normal allele could sometimes be triggered by azacytidine, indicating that the original 'mutation' was probably caused by methylation. Because revertants for the 'stable' imposition of methylation patterns might occur, variegation in phenotype might be anticipated for such traits. This would indicate that the original mutation had been at the level of methylation rather than gene sequence.

The most lasting impression to have been made on my mind at this symposium is likely to be the sense that intellects have been challenged by observations that cannot yet be explained: however, watch this space! Yet further interactions between clinical observation, scientific hypothesis, and experiment will be needed for the understanding of imprinting to be achieved.

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