# Know Your Chromosomes

## 3. Hybrid Cells and Human Genetics

*Vani Brahmachari*

**The selective elimination of human chromosomes in mouse-human hybrid cells generates a unique system for cytogenetic analysis. The use of such somatic cell hybrids in chromosome mapping is discussed in this part of the series.**

**Vani Brahmachari is at the Developmental Biology and Genetics Laboratory at Indian Institute of Science. She is interested in understanding factors other than DNA sequence *per se*, that seem to influence genetic inheritance. She utilizes human genetic disorders and genetically weird insect systems to understand this phenomenon.**

Thanks to our unique abilities we are capable of reigning over almost all living beings: certainly over mice, rats and hamsters. However, the results of artificially fusing a mouse cell with a human cell suggest otherwise. When two cells are fused under suitable conditions, their cytoplasms get mixed first, followed by the fusion of the two nuclei. After nuclear fusion, as the cells continue to divide, some of the human chromosomes are selectively lost while all the mouse chromosomes remain intact. As if compromising on coexistence, a certain number of human chromosomes, varying from one to five pairs, remain stable in these hybrid cells after several divisions. In spite of this chromosome elimination we have exploited hybrid cells to learn about our genetic endowment!!

### Exploiting The Hybrids

The basic scheme for generating hybrid cells is shown in *Figure 1*. We have already learnt that human chromosomes can be identified by their staining properties and the specific genes they contain. Therefore it is not difficult to imagine that by careful experimentation one can create a library, not of books, but of hybrid cells. Each clone of cells[1] in the library, would contain only one or a pair of human chromosomes, plus a background of mouse chromosomes. Cell fusions have been carried out not only between human and mouse cells but also between human and

[1] A clone of cells derived from a single parent cell is expected to consist of cells identical in most respects.

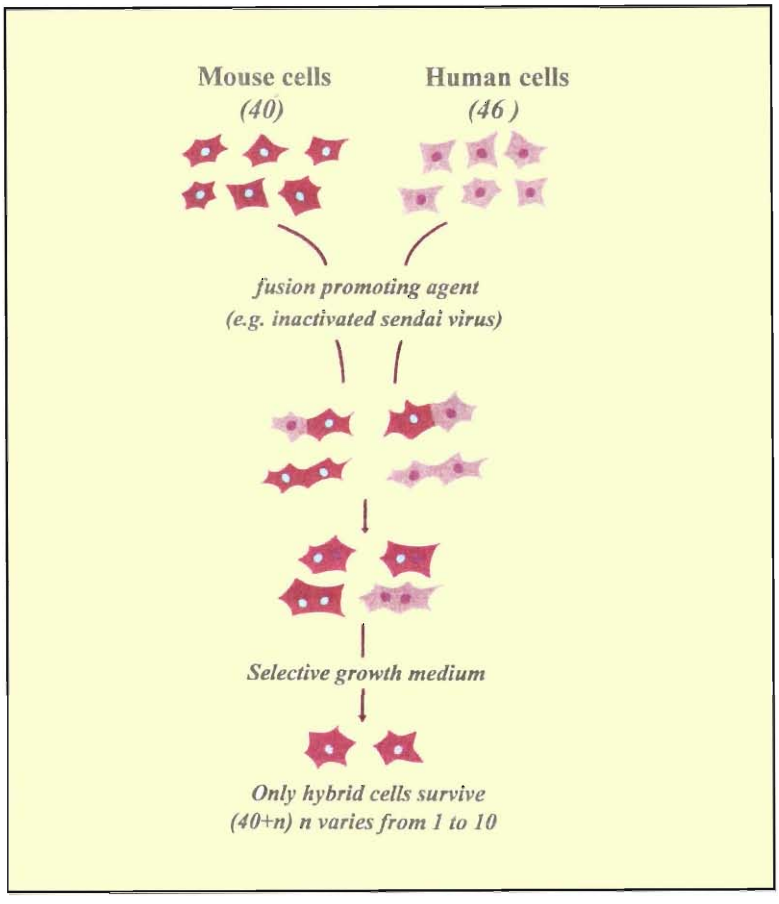



***Figure 1 The basic scheme for generating hybrid cells.***

rat cells. In all these instances, there is selective loss of human chromosomes. The reasons for this are not known. Human cells can be fused among themselves too, but this does not offer any specific advantage in gene mapping. How do hybrid cells help in mapping a gene? Basically one should be able to assess the presence of the human genes either bv their function or by physically identifying them. I will illustrate both of these approaches with a specific example.

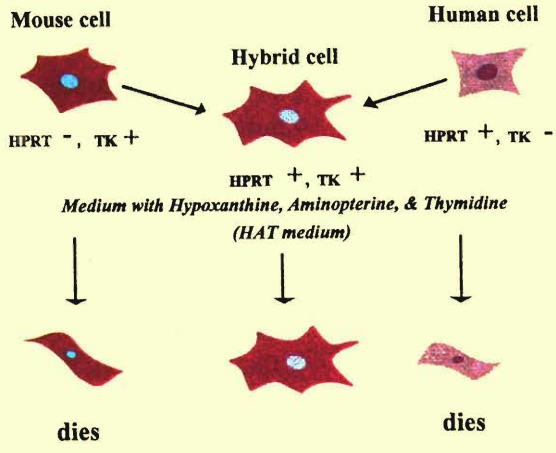
Each clone of cells in the library would contain only one or a pair of human chromosomes, plus a background of mouse chromosomes.

## Tracking By Function

Central to this approach is the possibility that the presence of a human chromosome can complement a known defect in the mouse cell. In order to select a hybrid cell of this nature one needs

### Selection of Hybrids by Function

Purines like guanine and adenine found in RNA and DNA are synthesized from a combination of simple precursors through several enzymatic reactions. This process is called *de novo* synthesis. Aminopterine (a drug), inhibits *de novo* synthesis. Under such conditions vertebrates utilize a salvage pathway to synthesize nucleotide triphosphates. Two key enzymes of this pathway are hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT) and thymidine kinase (TK).




When aminopterine inhibits *de novo* synthesis, the mouse cells cannot utilize hypoxanthine as they are $HPRT^-$ and similarly human cells cannot utilize thymidine as they are $TK^-$. But hybrid cells (shown in pink) survive as they have both enzymes; TK from mouse and HPRT from human cells. By karyotyping hybrid cells, it is seen that they have the human X-chromosome. Thus one can conclude that HPRT gene is on the X-chromosome.

to devise a condition in which only the hybrid cells but not the parent cells (namely the mouse and the human cells) survive. This is done by starting with parent cells which are each defective in one of two different enzymes and therefore can survive only in a set of conditions, say growth medium A. But when a hybrid is formed the defects in the two parent cells are compensated or complemented by each other and hence the hybrid can survive in a growth medium B, where the parent cells cannot survive. This is illustrated in the box above.

Having selected the hybrid cell one can propagate it and analyse its chromosomal profile or karyotype. There is a lucky break here! All mouse chromosomes are *acrocentric*, meaning that the centromere is at one end and they look like the letter 'V' in a

How do hybrid cells help in mapping a gene? Basically one should be able to assess the presence of the human genes either by their function or by physically identifying them.

metaphase preparation; this makes them distinct from human chromosomes. We also know (***Resonance***, Vol.1, No.1, January 1996) that one can identify each human chromosome with a specific banding pattern.

Using this approach, one selects for hybrid cells containing the human chromosome bearing the gene that can complement the deficiency in the mouse cell. For instance, mouse cells defective in enzyme E1 and human cells defective in enzyme E2 are chosen as parent cells. Hybrid cells grow in the special growth medium provided they have enzyme E1 coded by the human chromosome along with the complete complement of the mouse genome. Thus one concludes that the human chromosome retained in these hybrid cells has the gene coding for enzyme E1.

With a combination of methods, it is possible to localize a gene not only to a chromosome, but also to a specific band on the chromosomes.

As you can see there are several conditions to be fulfilled before you localize a gene to its chromosome by this approach. The major criteria are (a) availability of parent cells with appropriate deficiencies and (b) a selective medium where only hybrid cells but not the parent cells grow.

Therefore the approach of functional complementation is of limited applicability and has been used to localize enzyme coding genes on chromosomes such as 17, 16, 12 and the X–chromosome. The other approach requires a knowledge of the DNA sequence of at least part of the gene. An analysis based on antibodies specifically directed against the human protein suspected to be expressed by the hybrid cell can also be used for selection.

## Mapping by DNA Sequence

Let us assume that we have a DNA fragment of known or unknown function from the human genome. Our aim is to localise this DNA fragment to a specific chromosome. One can have a library of hybrid cells, say from 1 to 23, each retaining one human chromosome. What one does is tag the DNA fragment on

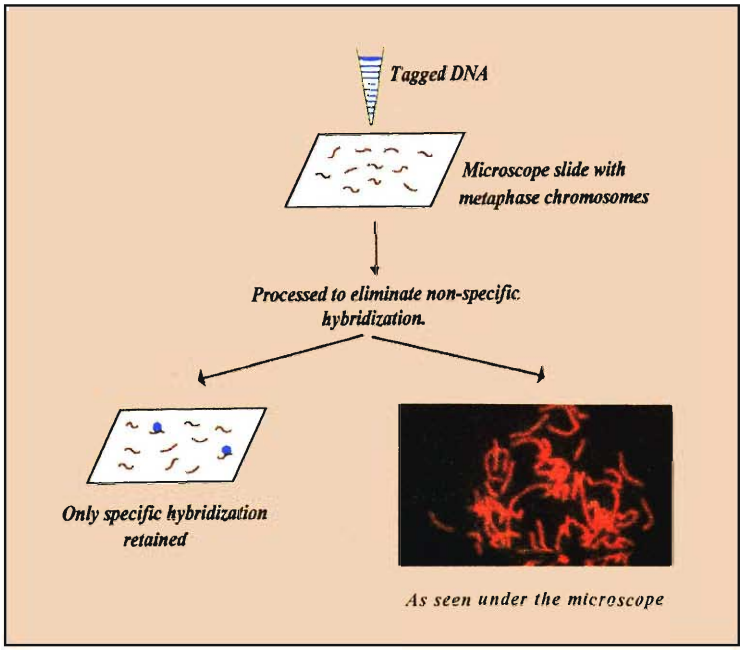



***Figure 2*** **In situ *hybridization; the photograph shows the view as seen under the microscope.***

hand either with a coloured chemical or a radioactive isotope. This tagged DNA can pair only on the chromosome where an identical DNA stretch is present. This is schematically depicted in *Figure 2*. This process is called *hybridization* and can be carried out either on chromosomes or on DNA derived from the clones. When it is done on a chromosome, it is called *in situ* hybridization. The same procedure can be carried out on chromosomes arrested at the metaphase stage of mitosis, from human cells as well.

With a combination of methods, it is possible to localize a gene not only to a chromosome, but also to a specific band on the chromosome. For instance, by hybridizing a DNA sequence corresponding to the interleukin (a cell growth factor) coding gene on metaphase chromosomes one can detect hybridization to the long arm of chromosome 4 between band 26 & 27. Thus the map position of the interleukin coding gene is denoted as 4q26-q27. Mapping genes by *in situ* hybridization on metaphase chromosomes of hybrid cells has become almost an obligatory step in human genome mapping.

Mapping genes by *in situ* hybridization on metaphase chromosomes of hybrid cells has become almost an obligatory step in human genome mapping.

**Table 1: A Listing of Representative Loci Mapped to Chromosomes 2, 3 and 4**

| Gene/disorder | Chromosomal location | *Mode of inheritance* |
|---|---|---|
| 1. Apolipoprotein B (APOB) | 2p24-p23 | *Autosomal dominant* |
| APOB is the main apolipoprotein of low density lipo proteins (LDL) that occurs in plasma. Deficiency leads to coronary artery disease, gait disturbance, ataxic hand movement. | | |
| 2. Colon cancer, familial (FCCI) | 2p16 | *Autosomal dominant* |
| The gene in this region is involved in repairing errors in DNA replication. Mutation results in failure of a repair system which leads to DNA instability and colon cancer. | | |
| 3. Pulmonary surfactant Apoprotein (PSP-B) | 2p12-p11.2 | *Autosomal recessive* |
| The gene codes for a protein associated with lipid rich pulmonary surfactant that prevents lung collapse by lowering surface tension at air-liquid interface. Defect in the gene leads to respiratory failure. | | |
| 4. Xeroderma pigmentosum II (XP2) | 2q 21 | *Autosomal dominant* |
| The product of this gene is a helicase involved in repairing DNA damage caused by ultraviolet radiation. A defective gene leads to sensitivity to ultraviolet rays and increases the predisposition to skin cancers. | | |
| 5. Insulin-dependent diabetes | 2q | *Autosomal recessive* |
| The nature of the gene is not known. Mutations in this region increase the susceptibility to insulin-dependent diabetes melitus. | | |
| 6. Brachydactyly Type E (BDE) | 2q 37 | *Autosomal dominant* |
| Mutations in this locus lead to short stature, shortening of fingers and reduction in number of digits. The kind and intensity of defects vary between the members of the same affected family. It is a locus which seems to be involved in a complex phenomenon like three dimensional form. | | |
| 7. Von Hippel-Lindan syndrome (VHL) | 3p26-p25 | *Autosomal dominant* |
| The syndrome is characterised by several carcinomas, renal cysts and hypertension. The nature of the gene(s) is not known. | | |
| 8. Hypernephroma (HN) | 3p 14.2 | *Autosomal dominant* |
| Mutations at this locus result in hereditary renal cancer, adenocarcinoma of the kidney. The nature of the gene(s) is unknown. | | |
| 9. Protein S (PSA) | 3p11.1-q11.2 | *Autosomal dominant* |
| It is a vitamin-K dependant plasma protein that prevents blood clotting. The deficiency in protein K results in thrombosis or inappropriate clotting of blood. | | |

| Gene/disorder | Chromosomal location | *Mode of inheritance* |
|---|---|---|
| 10. Rhodopsin (RHO) | 3q21-q24 | *Autosomal dominant and recessive forms known* |
| This is a visual pigment mediating vision in dim light. Defect results in retinitis pigmentosa, defects in retinal pigmentations and night blindness. | | |
| 11. Sucrose-isomaltase (SI) | 3q25-q26 | *Autosomal recessive* |
| It is an enzyme found in small intestine brush-border membrane, involved in hydrolysing sucrose. Deficiency of the protein results in malabsorption of sucrose from the diet leading to diarrhoea, disaccharide intolerance, and kidney stones. | | |
| 12. Huntington Chorea (HD) | 4p 16.3 | *Autosomal dominant* |
| The disease gives rise to progressive, selective neural cell death associated with choreic movements and dementia. It is associated with CAG triplet repeat expansion in a gene called huntingtin. | | |
| 13. Cyclic nucleotide gated channel Photoreceptor (CNCG) | 4p14 | *Autosomal dominant and recessive forms known* |
| Involved in the function of rods and cones in the eyes. Defective protein leads to retinitis pigmentosa. | | |
| 14. Dysalbuminemia (DALB) | 4q11-q13 | *Autosomal dominant* |
| Gene codes for albumin which is one of the most abundant proteins of blood serum. It acts as a carrier for steroids, fatty acids and thyroid hormones. Mutations in this gene result in disorders of connective tissue like cartilage, tendon and ligament. | | |
| 15. Mucolipidosis II (ML2) | 4q 21-q23 | *Autosomal recessive* |
| The disorder is characterised by congenital dislocation of the hip, thoracic deformities, hernia, slower psychomotor development and restricted joint movements. It is suspected that there is leakage of enzymes from lysosomes, the suicide bags of the cell. | | |
| 16. Interleukin-2 (IL2) | 4q 26-q27 | *Autosomal recessive* |
| It is a cell-growth factor required for the proliferation of lymphocytes. Defect in the gene results in severe combined immunodeficiency. | | |
| 17. Muscular dystrophy Facio scapula humeral (FSHD) | 4q 35-qter | *Autosomal dominant* |
| The disorder leads to muscle weakness; symptoms appear early in infancy first in the face, upper arms and shoulder muscle. Gene responsible not identified. | | |

## Genes on Chromosomes 2, 3 and 4

A representation of chromosomes 2, 3 and 4 is shown *in Figure 3*. As of now the total number of genes localized to each of these is: 199 (chromosome 2), 191 (chromosome 3) and 150 (chromosome 4). These genes include those responsible for encoding enzymes, growth factors and proteins involved in neural pathways. *Table 1* lists some genes from chromosomes 2, 3 and 4. As one may notice, there is no relationship between the chromosomal location of genes and their function. In most cases, there is no clustering of genes just because they are part of the same metabolic pathway.

Huntington's disease is an example in which the severity of the disease depends on the sex of the parent from whom the defective gene is inherited.

One of the genes mapped to chromosome 4 is the gene for Huntington's disease or Huntington's chorea. Named after George Huntington, a physician who described the disorder in 1872, it is a dominant autosomal disorder that leads to nerve cell death, progresses with age and is associated with rigidity, loss of memory and personality changes. Typically, the patients die 10-15 years after the onset of the disease. This disorder is representative of a class of disorders which may not be seen at birth, but occurs at different ages in different patients. The age of onset of the disease can be from 10 to 70 years. This is also an example in which the

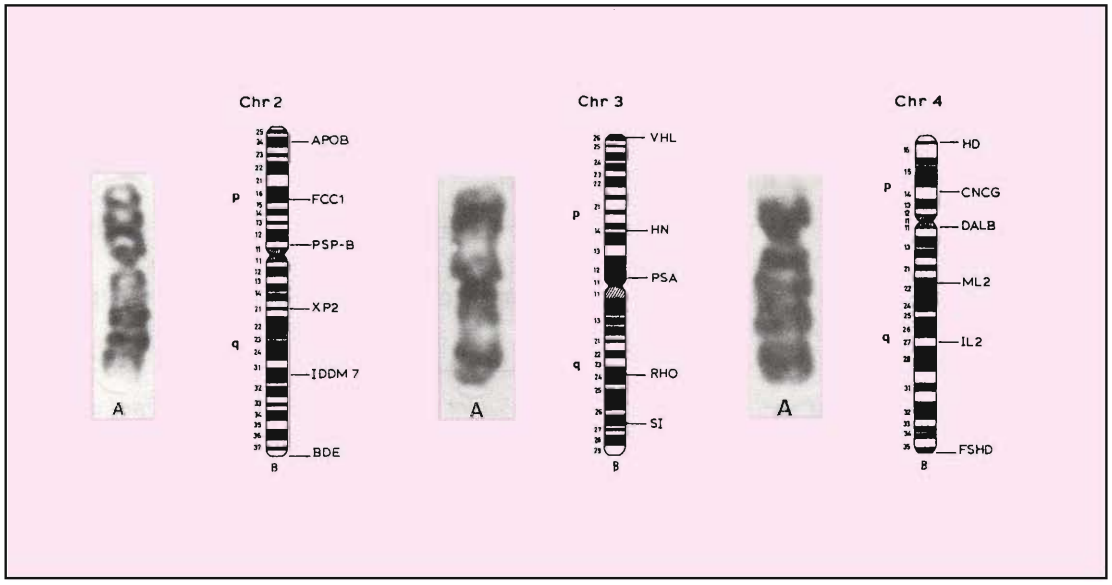



***Figure 3 A representation of chromosomes 2, 3 and 4.***

severity of the disease depends on the sex of the parent from whom the defective gene is inherited. If the child inherits the defective gene from the father, it is likely to have a more severe disorder than the father and at an age earlier than him. When inherited from the mother, both severity and age of onset are likely to be simillar between the child and the mother.

*Address for correspondence*
Vani Brahmachari,
Developmental Biology and
Genetics Laboratory,
Indian Institute of Science,
Bangalore 560 012, India.

The identification of the gene responsible for Huntington's disease was announced in 1993 in a research paper authored by 58 scientists belonging to six different groups! The protein coded by the gene is named 'huntingtin' and is believed to exert its effects by interacting with other proteins. The nature of the mutation that leads to the disorder has helped us understand at least partially, the basis of differences in severity and age of onset from one generation to the other. But how the human system tolerates the absence of a functional gene product in early life but not later is far from clear. Perhaps there is functional redundancy suggesting that nature, the excellent designer, has provided sufficient backup to avoid a system breakdown.

## Suggested Reading

Nils R Rigertz and Robert E Savage. Cell Hybrids. Academic Press. New York, San Francisco, London. 1976.

Daniel Hartl. Human Genetics. Harper and Row Publishers. New York, Cambridge, London. 1983.

Friedrich Vogel and Arno G Motulsky. Human Genetics: Problems and Approaches. Springer - Verlag. Berlin, Heidelberg, New York, Tokyo. 1986.

Victor A McKusick. Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive and X linked Phenotypes. Vol I and II Tenth Edition. The Johns Hopkins University Press. Baltimore and London. 1992.

**Anonymous poetic supplication ...**

*Grant, Oh God, thy benedictions*
*On my theory's predictions,*
*Lest the facts when verified,*
*Show Thy servant to have lied.*