Know Your Chromosomes

1. Nature's Way of Packing Genes

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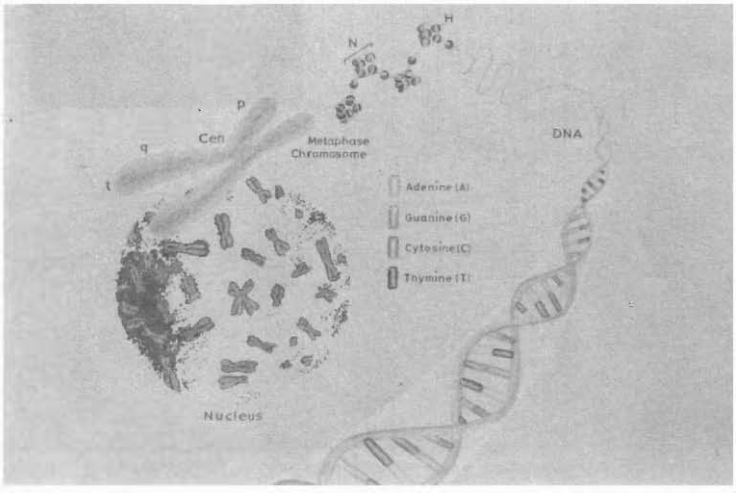
Vani Brahmachari is at the Developmental Biology and Genetics Department at Indian Institute of Science. She is interested in understanding factors other than DNA sequence *per se*, that seem to influence genetic inheritance. She utilizes human genetic disorders and genetically weird insect systems to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

The study of cellular structures including chromosomes began as early as the 17th century. The organization of chromosomes, the structure and function of genes and the role of genetic mutations in diseases continue to be an area of intense scientific investigation.

The size of an average human cell is 20-40 micrometers (μm) or microns (μ). One micrometer is one millionth of a meter i.e., 10^{-6} meters). Deoxyribonucleic acid (DNA) the primary genetic material is located in the nucleus which is 8-20 μm . The DNA present in a single human cell if stretched out completely would have a length of about 1.8 meters (6 feet). That leaves us with the puzzle of how cells pack up 1.8 meters of DNA inside a tiny sac like the nucleus which is 0.000020 meters in diameter!! Nature has divided this DNA into 23 pieces and compacted them several fold to accommodate them in the cell nucleus. These pieces of DNA which are clusters of several genes are called linkages groups or chromosomes. Therefore chromosomes are nothing but long



SANJEEVA NAYAK

Figure 1 From chromosome to DNA.. 't' is telomere; the end of a chromosome; 'q'- longarm; 'Cen' is centromere, which aids in segregating chromosomes to daughter cells during cell division; 'p'- short arm; 'N'- nucleosome, the unit of organization of chromosomes; 'H'- histones, which are proteins present in nucleosomes as octamers around which approximately 150 base pairs of DNA are wrapped. Adenine, Guanine, Cytosine and Thymine are nitrogen containing bases present in DNA.

stretches of DNA compacted with the help of proteins. Under an electron microscope, chromatin appears as beads on a string: the string being DNA and the beads being the proteins (*Figure 1*).

In the 17th century, the structure of various cell types was analysed by light microscopy using specific dyes or stains. The cellular structures which readily took up the stain were the complexes of DNA and protein. Since the chemical nature of the darkly stained bodies was not known they were simply called *'chromatic elements'* — meaning coloured elements. The term chromosome was suggested by W Waldeyer in 1888. The number of chromosomes in a given species is characteristic of that species, and is maintained constant from one generation to the next. The chromosome numbers of some plant and animal species are listed in *Table 1*. Most organisms are 'diploid' meaning that they have two copies of each chromosome, one received from the father and the other from the mother. The sperm and the egg nuclei (which fuse during fertilization to form the zygote, that grows and develops into a complete organism) contain only a single copy of each chromosome. Therefore sperms and eggs are said to be 'haploid'. For instance, the diploid number of chromosomes in humans is 46 and therefore the haploid number is 23. The 46 chromosomes in each of our cells carry all the genetic information necessary to build a human being, in the form of genes made up of DNA.

Table 1
Organism with its Chromosome number*

Man	46
Chimpanzee	48
Dog	78
Donkey	62
Mouse	40
Frog	26
Carp	104
Silk worm	56
Fruit fly (Drosophila)	8
Rice	24
Wheat	42
Tomato	24
Pea	14

**The number indicates the diploid or 2n number of chromosomes. One chromosome of each pair is received from the mother and the other from the father.*

Chromosomes: The Vehicles of Heredity

The number of chromsomes in humans has been known for only 39 years, while Mendel formulated his laws of inheritance 130 years ago and his work was rediscovered almost 95 years ago. We have been aware of the fact that children take after their parents and that certain diseases run in families. Plant breeding has been successfully practised by farmers who did not understand the genetic basis for crop improvement. Gregor Mendel, regarded as the father of genetics, saw a pattern in the inheritance of distinct characters in pea plants. The deliberate design of crosses between

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50×10^{-6} m		Cell: is the building block of all plants and animals. There are about 100 trillion cells in the human body.
20×10^{-6} m		Cell nucleus : is present within each cell except red blood cells in humans and other mammals. This contains the genetic material, DNA organized as chromosomes. In each of our cells there is about 6 feet long DNA packed into 46 units called chromosomes.
6×10^{-6} m		Chromosome: is a long thread of DNA wrapped around proteins. A specific block of DNA represents a gene.
34×10^{-9} m		Gene: is a unit of information usually containing information to make a protein. There are about 50,000 - 1,00,000 genes in each human cell.
$3\text{-}5 \times 10^{-9}$		Proteins: are workhorses of the cell serving various purpose like transport of ions, antibodies to fight infections, and as catalysts in various biochemical reactions.

SANJEEVA NAYAK

pea plants with distinct characters led him to formulate the laws of inheritance. The first application of Mendel's 'gene' concept to a human trait was by the physician A Garrod. He described the genetic disease alkaptonuria *(Box 2)* as an alteration in specific biochemical reactions leading to the excretion of homogentisic acid in urine. He introduced the concept of 'chemical individuality' and observed that an individual either does or does not excrete homogentisic acid; no patient exhibits intermediate states. In other words, the trait is a discrete one. This defect occurred in

Alkaptonuria

Amino acids are primarily used as building blocks for proteins and as precursors for other biomolecules like hormones, purines and pyrimidines. When an excess of protein is ingested, amino acids derived from protein degradation are used as a source of energy by a process called oxidative degradation.

In one such pathway phenylalanine is converted into acetoacetyl COA through a series of enzymatic reactions.

One of the steps in this pathway is the conversion of homogentisic acid, an intermediate in this pathway, to 4-malelyl acetoacetic acid by an enzyme called homogentisic acid 1,2-dioxygenase.

If an individual has a defect in the gene coding for this dioxygenase it will lead to the production of a non-functional enzyme. This in turn results in the accumulation of homogentisic acid and its excretion in urine. This condition is described as alkaptonuria.

Defects at other steps in this pathway lead to genetic disorders like phenylketonuria, tyrosinemia and albinism.

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children of several first-cousin marriages but not all marriages between relatives resulted in children with the disorder. He reasoned that there may be some peculiarity in the parents of children who inherited the disease. Garrod recognised that Mendel's laws of heredity could provide a reasonable basis for the phenomenon. In 1908, he published his monograph on inborn errors of metabolism which was a reflection of his great insight into the role of genetics in human physiology. As is often the case in the history of science, Garrod's contributions to human genetics remained unappreciated during his lifetime.

Before the rediscovery of Mendel's work in 1900, the process of

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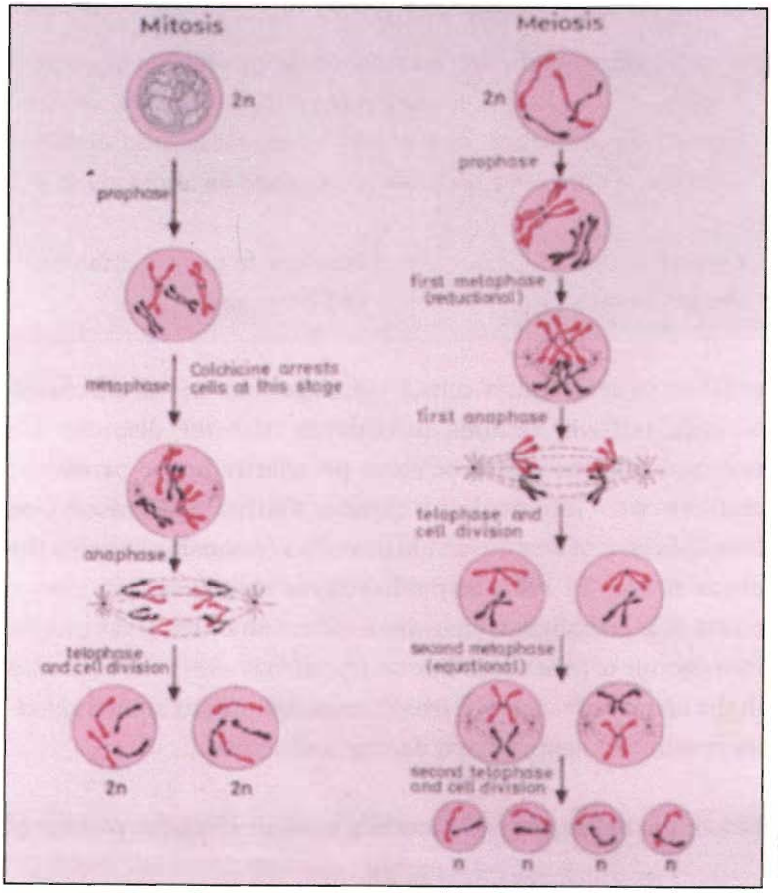
cell division, meiosis and mitosis (*Figure 2*) had been analysed and the chromosomes were identified as entities that are evenly distributed between daughter cells during cell division. The similarity between Mendelian segregation and chromosomal distribution during meiosis was correlated and chromosomes were identified as bearers of genetic information. Soon after the rediscovery of Mendel's work (1902 and 1903) chromosomes were recognised as units of heredity by different scientists independently. Thus the discipline of cytogenetics developed with experiments in plants like Lilium and insects like the fruit fly, Drosophila. Although it was only in 1956 that the correct number of chromosomes in humans was established, the Mendelian mode of inheritance was illustrated by the inheritance of the ABO blood groups by Landsteiner in 1900 and by two German scientists in 1911.



SANJEEVA NAYAK

Figure 2 Diagrammatic representation of steps in nuclear division during cell division. 2n and n represent the diploid and haploid state of the nuclei. In the given example 2n = 4. Meiosis takes place during production of the egg and the sperm.

In the summer of 1955 Albert Levan, a Swedish cytogeneticist visited T C Hsu, who had developed a modified method for chromosome preparation and learned the method of preparing chromosomes from human cells. Later, Albert Levan with Joe Hin Tijo discovered that by adding colchicine, an alkaloid derived from plants, the highly condensed state of metaphase chromosomes can be blocked from proceeding further (*Figure 3*). The tissue with which they worked was human embryonic liver. Out of the 261 metaphase cells they observed most had 46 chromosomes. To this day a large number of metaphases are observed by cytogeneticists before reporting the diagnosis of the chromosomal status of a patient. Following this discovery it was found that what was described as Mongolism and later as Down's Syndrome was actually the presence of three copies of chromosome 21 in the patients instead of the normal two. Anomalies in chromosome number, in particular that of the sex chromosomes, were also reported in patients who had abnormal sexual development. All these were substantiated after the development of new meth ds for the analysis of human chromosomes. From this perspective the revolution in the study of chromosomes referred to as cytogenetics seems to have arisen from methodological improvements rather than the development of a new concept. The advantage was that inferences drawn from previous observations did not lose their value but got further supplemented and reinforced. Thus human cytogenetics attained a new dimension. In the following years it was discovered that several human hereditary disorders are due to chromosomal defects.

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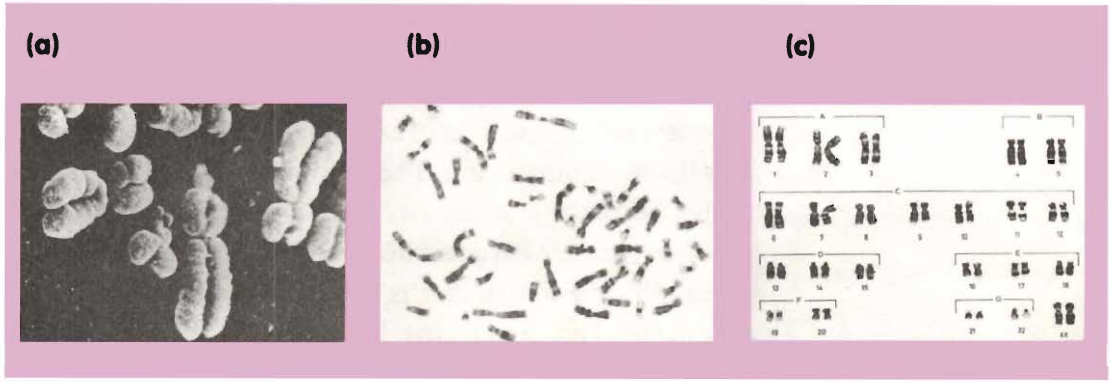
***Figure 3** Chromosomes as visualised by different methods (a) scanning electron micrograph of human chromosomes. (b) a metaphase spread prepared from lymphocytes (WBC) at metaphase stage of cell division. The banded pattern is due to differential giemsa staining. (c) a metaphase spread photographed under the microscope; individual chromosomes are cut up and arranged in order after comparison with a standard banding pattern. This is called a* karyotype. *This karyotype (provided by Dr Sridevi Hegde, St John's Medical College, Bangalore) is that of a woman.*



SRIDEVI HEGDE

The procedure of preparing a picture of chromosomes of an individual is called 'karyotyping'.

Chromosomal Nomenclature

The chromosome number in each cell was established but how was the nomenclature arrived at? In 1968 it was realised that certain dyes stain chromosomes in a non-uniform fashion giving rise to lighter regions and darkly stained regions. This produces the pattern shown in *Figures 3 b,c*. There is also a variation in the length of chromosomes and in the position of the centromere which helps in the segregation of chromosomes to daughter cells, during cell division. Chromosomes are ordered and numbered by two different conventions. Based on their length they are ordered 1 to 23. In the other system 23 pairs are distributed into groups A to G based on their length and the centromere position. Both systems are indicated in *Figure 3c*, the pairs from 1 to 22 are called autosomes and the 23rd pair is called the sex chromosome, typically denoted by the letter X and Y. A female has 22 pairs of autosomes and two X-chromosomes (44XX), whereas a male has 22 pairs of autosomes, an X-chromosome and a Y-chromosome (44XY). Now you can appreciate the fact that by a chromosomal analysis of an individual one can identify any change in number, length or staining pattern of chromosomes. This procedure of preparing a picture of chromosomes of an individual is called 'karyotyping'.

From Chromosome to Genes

The total amount of DNA in 23 chromosomes is estimated to be three billion (3×10^9) base pairs. Base pair means a pair consisting of adenine and thymine (A-T) or guanine and cytosine (G-C) — the nitrogen containing bases in the building blocks of DNA. Therefore the total amount of DNA in our cell is six billion base pairs (in 46 chromosomes). The identification of any change in the number of chromosomes is relatively easy, but any change or loss of only one or a few genes from a chromosome will not lead to a change in the length that is detectable under a microscope normally used for karyotyping. For instance, to find the cause of a hereditary disorder like haemophilia (a genetic disorder result-

ing in defective blood clotting), the challenge before the scientists was to find a single base pair change out of six billion base pairs. It sounds almost impossible. But scientists have worked out a way of narrowing down the region of the defect step by step. Some of the steps in this process are as follows: (a) to derive the pattern of inheritance (autosomal or sex linked) by family history or pedigree analysis; (b) to find the linkage group or the chromosome on which the gene is likely to be located; (c) to find neighbouring markers or genes and (d) finally to find the defective gene itself. With the advent of modern methods in biology, the order in which the steps are taken towards identifying a gene related to a trait can be different in different cases. But whatever the starting point one would like to derive all the information outlined in (a)-(d) to help in diagnosis, treatment or prevention of a genetic disorder or in finding a gene.

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The total number of genes known in humans to date is reaching 6,000. But this is only about 6 to 12% of the total number of genes estimated to be present in humans. Moreover each gene does not function in isolation. It is like the words in a sentence; with different meanings in different contexts. Similarly, a gene can be a part of different complex processes contributing to different end products. Most often it is the defect in a gene which leads to the understanding of its normal function. In future articles, we will learn more about the structure and function of individual chromosomes along with the processes used to study them.

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Suggested Reading

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