Eeva Therman

Human Chromosomes

Structure, Behavior, Effects

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With 55 Figures

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Preface

This book provides an introduction to human cytogenetics. It is also suitable for use as a text in a general cytogenetics course, since the basic features of chromosome structure and behavior are shared by all eukaryotes. Because my own background includes plant and animal cytogenetics, many of the examples are taken from organisms other than man. Since the book is written from a cytogeneticist's point of view, human syndromes are described only as illustrations of the effects of abnormal chromosome constitutions on the phenotype. The selection of the phenomena to be discussed and of the photographs to illustrate them is, in many cases, subjective and arbitrary and is naturally influenced by my interests and the work done in our laboratory.

The approach to citations is the exact opposite of that usually used in scientific papers. Whenever possible, the latest and/or most comprehensive review has been cited, instead of the original publication. Thus the reader is encouraged to delve deeper into any question of interest to him or her.

I am greatly indebted to many colleagues for suggestions and criticism. However, my special thanks are due to Dr. JAMES F. CROW, Dr. TRAUTE M. SCHROEDER, and Dr. CARTER DENNISTON for their courage in reading the entire manuscript. I wish to express my gratitude also to the cytogeneticists and editors who have generously permitted the use of published and unpublished photographs.

Members of my laboratory have been very helpful in the process of putting this book together. Mrs. BARBARA SUSMAN has been involved in all the phases, from compiling reference lists to designing illustrations.

The photographic work has been done by Mr. WALTER KUGLER, Jr.; our excellent secretary, Ms. CHRISTINE ONAGA, has cheerfully typed and retyped the manuscript.

However, my most special thanks I wish to reserve for the editor of this book, Ms. MARY LOU MOTL. When my Finnish-English emerges from her competent hands, it resembles the text I would have liked to have written in the first place.

Madison, Wisconsin
Septe.mber, 1980

EEVA THERMAN

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Past and Future of Human Cytogenetics

The Past of Human Cytogenetics

Before 1956 two "facts" were known about human cytogenetics. The human chromosome number was believed to be 48 and the XX-XY mechanism of sex determination was assumed to work in the same way as it does in *Drosophila*. Studies of the fruit fly had shown that the *ratio* of the number of X chromosomes to the number of sets of autosomes determines the sex of the organism. Both these fundamental notions about human chromosomes were eventually proved wrong.

The year 1956 is often given as the beginning of modern human cytogenetics, and indeed the discovery of Tjio and Levan (1956) that the human chromosome number is 46, instead of 48, was the starting point for subsequent spectacular developments in human chromosome studies. The difficulties of writing about even fairly recent history are well demonstrated by the very different accounts of this discovery related by the two participants themselves (Tjio, 1978; Levan, 1978).

The history of human cytogenetics has been reviewed at length several times, for instance, by Makino (1975) and recently by Hsu (1979). Hsu's delightful book relieves this author of the responsibility of giving a detailed description of the developments in the field; instead I will simply outline the major events in cytogenetics.

Hsu (1979) divides human cytogenetics conveniently into four eras: the dark ages before 1952, the hypotonic period from 1952 to about 1958, the trisomy period between 1959 and 1969, and the chromosome banding era that started in 1970 and still continues. In the following discussion only a few highlights of these stages will be recounted.

The Dark Ages

The difficulties faced by the early cytogeneticists are illustrated by a comparison of Fig. III.1 with the other photomicrographs of human chromosomes in this book. Despite the lack of clarity, the lymphocyte mitosis in Fig. III.1 shows the chromosomes considerably better than did the slides of paraffin-sectioned testes, stained with hematoxylin, that were used during the first quarter of this century. Of these studies only the paper by Painter (1923) is mentioned here, since it determined the ideas in this field for the next 33 years. Even though Painter's report that the human chromosome number was 48 was worded quite cautiously, the more often it was quoted, the more certain the finding seemed to become.

Despite the primitive techniques available, the groundwork for future studies was laid during the dark ages. The first satisfactory preparations of mammalian chromosomes were obtained by squashing ascites tumor cells of the mouse (Levan and Hauschka, 1952, 1953) and of the rat (cf. Makino, 1975). The first successful prefixation treatment with chemical substances was performed on mouse tumor cells by Bayreuther (1952); later colchicine or its derivatives were used.

During this era, mammalian tissue culture techniques were developed. Prefixation treatment with hypotonic salt solution, which swells the cells and thus separates the chromosomes, was a decisive improvement in cytological technique. The hypotonic treatment was launched by Hsu (1952), although other laboratories were experimenting with similar treatments at the same time.

The Hypotonic Era

The simultaneous use of a number of new techniques finally made it possible to establish the right chromosome number in man. They were the tissue culture and squash techniques combined with treatments with colchicine and hypotonic solution prior to fixation. Before the end of 1956, the finding of Tjio and Levan in embryonic lung cells was confirmed in human spermatocytes by Ford and Hamerton (1956) whose photomicrographs also showed that the X and Y chromosomes are attached end-to-end by their short arms in meiosis. During the hypotonic era the analysis of the human karyotype was also begun.

The Trisomy Period

The new techniques were soon applied to chromosome analyses of individuals who were mentally retarded or had other congenital anomalies or both. The first autosomal trisomy was described by Lejeune et al (1959) who found that mongolism (Down's syndrome) was caused by trisomy for one of the smallest human chromosomes. During the same year it was reported that Turner's syndrome was characterized by a 45,X

chromosome constitution (Ford et al, 1959) and Klinefelter's syndrome by a 47,XXY chromosome complement (Jacobs and Strong, 1959). In addition, the first XXX woman was described (Jacobs et al, 1959). The observations on Turner's and Klinefelter's syndromes showed that the male sex in human beings is determined by the presence of the Y chromosome. Later it was established that the Y chromosome is effective in determining male sex even if it is combined with four X chromosomes; individuals with the XXXXY sex chromosome constitution are males, although abnormal.

The following year, D_1 trisomy (now known to be 13 trisomy) (Patau et al, 1960) and 18 trisomy (Edwards et al, 1960; Patau et al, 1960; Smith et al, 1960) were described. With these discoveries the viable autosomal trisomies seemed to be exhausted, although later the exceedingly rare 22 trisomy was found, and chromosome studies turned to structural aberrations and their phenotypic consequences.

These developments coincided with an important innovation in cell culture technique. Nowell (1960) and Moorhead et al (1960) launched the short-term culture technique using peripheral lymphocytes. The effectiveness of the technique was based on the mitosis-inducing ability of phytohemagglutinin. Such cultures, combined with the trick of drying the chromosomes directly on microscope slides (Rothfels and Siminovitch, 1958), are still the most important source of human and mammalian chromosomes.

Chromosome Banding Era

Despite all claims to the contrary, the chromosomes in groups B, C, D, F, and G could not be identified individually on morphological grounds (Patau, 1960); the numbers attached to the paired-off chromosomes in prebanding karyotypes represented sheer guesses. Although autoradiography had allowed the accurate identification of some chromosomes (cf. Patau, 1965), the degree of precision was increased by orders of magnitude with the introduction of chromosome banding techniques. In 1970, Caspersson et al applied fluorescence microscopy, which they had originally used to study plant chromosomes, to the analysis of the human karyotype. They discovered that the chromosomes consist of differentially fluorescent cross bands of various lengths. Careful study of these bands made possible the identification of all human chromosomes. This discovery was followed by a flood of different banding techniques that utilize either fluorescent dyes or the Giemsa stain. The banding of prophase chromosomes makes it possible to determine chromosome segments and breakpoints even more accurately (Yunis, 1976).

Another milestone was the discovery that chromosomes that incorporate bromodeoxyuridine (BrdU) instead of thymidine have different staining properties. This phenomenon has been successfully used to

reveal the late-replicating chromosomes and chromosome segments (Latt, 1974). It also provides the basis for the study of sister chromatid exchanges (Latt, 1973).

It is much more difficult to obtain satisfactory chromosome preparations of the male meiosis—not to mention the female meiosis—in man than, for instance, in the mouse. But lately these difficulties have to some extent been overcome. The early stages of meiosis have been analyzed successfully in the oocytes (e.g., Therman and Sarto, 1977; Hultén et al, 1978), whereas work on the spermatocytes has yielded clear photomicrographs of the later stages (e.g., Stahl et al, 1973).

Human Sex Chromosomes

Throughout Hsu's (1979) four eras the understanding of the function and behavior of the mammalian sex chromosomes increased steadily. One of the first important observations was that the neural nuclei of the female cat had a condensed body, missing in the male nuclei (Barr and Bertram, 1949). This body has been called sex chromatin, the Barr body, or X chromatin.

The single-active X hypothesis of Lyon (1961; cf. Russell, 1961) had a decisive influence on the entire field of mammalian sex chromosome studies. According to the Lyon hypothesis, as it is called, one X chromosome in mammalian female cells is inactivated at an early embryonic stage. The original choice of which X is inactivated is random, but in all the descendants of a particular cell the same X remains inactive. If a cell has more than two X chromosomes, all but one of them are turned off. This mechanism provides dosage compensation for X-linked genes because each cell, male or female, has only one X chromosome that is transcribed. The Barr body is formed by the inactive X chromosome (Ohno and Cattanach, 1962), which is out of step with the active X chromosome during the cell cycle.

One of the highlights in the study of mammalian sex determination is the recent discovery that the primary sex determination of the Y chromosome is mediated through the H-Y antigen, which is a plasma membrane protein (cf. Ohno, 1979). This antigen induces the development of testicular tissue, which in turn determines secondary sex development through its production of androgen.

Evolution of Human Chromosomes

The Phylogeny of Human Chromosomes, as Seuánez (1979) calls his book on the subject, has been studied intensively in recent years. A comparison of the chromosomes of man with those of his closest

relatives—the chimpanzee, gorilla, and orangutan—shows that 99 percent of the chromosome bands are shared by the four genera. The most prominent differences in banding patterns occur in the heterochromatic regions (cf. Seuánez, 1979). Surprisingly it appears that man's closest living relative is the gorilla and not the chimpanzee, as has been believed until now.

The similarity of the chromosome banding pattern in all four genera demonstrates that most of the individual bands have retained their identity for more than 20 million years, and many of them for considerably longer. A number of chromosomes in man and the great apes are identical. The most conservative of these chromosomes is the X, which has not changed in morphology, at least between the monkey and man. Its gene content is assumed to have remained the same throughout mammalian development, or for some 125 million years (cf. Ohno, 1967; Seuánez, 1979). The comparison of the chromosomes of man and his relatives is now under way on the molecular level too (cf. Jones, 1977).

Nomenclature of Human Chromosomes

As the number of laboratories involved in analysis of human chromosomes multiplied, so did the systems of chromosome designation. In an effort to create order in this threatening chaos, four conferences on chromosome nomenclature were held: in Denver (1960), London (1963), Chicago (1966), and Paris (1971) (cf. Makino, 1975). The recommendations of the latest conference (Paris Conference, 1971) which included the designations for the chromosome bands, are now in use (Chapter V).

The Future of Human Cytogenetics

The expansion of the science of human cytogenetics in somewhat more than 20 years is little short of miraculous; by now, from the viewpoint of cytogenetics, man is by far the most extensively studied organism. During its early stages, human cytogenetics was a more-or-less applied science: phenomena previously described in plants and animals were now being observed in man. However, human cytogenetics has come of age, and advances in this field have inspired studies in other branches of human genetics. Indeed it is the coordination of different approaches that has led to the most interesting results in this field. During the "dark ages", human cytogeneticists borrowed techniques from plant and animal studies. Now the opposite is often true, and both animal and plant chromosome studies owe a debt to the work done on humans.

Predictions of future developments in a scientific field can only be based on its present state. However, just as unexpected findings in the

past have changed the course of events, they will undoubtedly do so in the future. In the following discussion, those approaches to human cytogenetics that seem most promising to this author are briefly reviewed.

Structure of the Eukaryotic Chromosome

Banding techniques added a new dimension to the understanding of the longitudinal differentiation of human chromosomes (Chapter V and VI). The quinacrine-bright bands seem to contain more heterochromatin, whereas the dark bands are more gene-rich. The main locations of constitutive heterochromatin have been determined, and different types of heterochromatin distinguished on the basis of their staining properties. Immunofluorescent stains that are specific for different chromosome constituents seem to offer a promising approach.

Only a few years ago very little information existed on the fine structure of the eukaryotic chromosome. There was an almost total gap between our knowledge of chromosomes, as seen in the light microscope, and what was known about DNA in vitro. This gap is now gradually being bridged, and in the not too distant future an understanding of how the chromosome is built out of its constituents ought to emerge.

A chromatid consists of one double helix of DNA. Its structure is determined by the base ratios and the arrangement of the bases. This structure is reflected in the distribution of the different classes of histones and other proteins along the chromosomes; the location of the proteins in turn determines the visible banding pattern.

Chromosomal DNA has been studied in vitro with different techniques. Based on the speed of renaturation of isolated, sheared DNA, it has been divided into fractions containing highly repetitive, intermediately repetitive, or unique sequences. Another approach to the analysis of DNA is the fractionation of native double-stranded DNA by cesium salt-density gradients. This technique shows that minor components, so-called satellite DNAs, differ in their buoyant densities from the main bulk of DNA. Hybridization of the various isolated DNA fractions (or the RNAs coded from them) on chromosomes demonstrates that repetitive sequences, as well as various satellite DNAs, are mainly localized in the bands representing constitutive heterochromatin. However, the exact relationships of the DNA fractions, isolated by different methods to each other and to the chromosome bands, are still largely unknown.

Another unanswered question is: What role does the ubiquitous, but apparently inert, heterochromatin play in the cell? Further, what is the function of the great amount of DNA in eukaryotic chromosomes, which does not represent constitutive heterochromatin but is not being transcribed either? The base sequencing of DNA, now under way, may provide answers to some of these questions.