HUMAN CHROMOSOMES

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1978
ACADEMIC PRESS
London and
New York

ACADEMIC PRESS INC. (LONDON) LTD
24/28 Oval Road
London, NW1

U.S. Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue,
New York, New York 10003

Library of Congress Catalog Card Number: 72-84358

ISBN: 0-12-262 150-6

Printed in Great Britain by
The Whitefriars Press Ltd., London and Tonbridge

Preface

This book is intended for anyone who is interested in human chromosomes; in what they look like, of what they are made, what they are for, and how they work. The median reader aimed at is the student of medicine, undergraduate or postgraduate, but I have tried to write as plainly as possible in an attempt to make the book comprehensible to anyone with some background in biology and chemistry. At the same time I hope that more senior research workers will find the book to be a useful compendium of up-to-date information and references.

This is not directly intended to be a textbook of medical cytogenetics; it does not deal specifically with the medical aspects of all chromosomal disorders; there are other books which already do this. Nor is it a comprehensive treatise on human cytogenetics; J. L. Hamerton's great two-volume work "Human Cytogenetics" is likely to remain the standard work on the subject for some years. This book is primarily concerned with the principles underlying the study of human chromosomes, and with trying to give an answer to the question "What may be gained by studying the human chromosomes in relation to such-and-such a problem?". It is true that a good deal of medical cytogenetics has crept in during the course of consideration of such principles; and in the final chapters there is some speculation on the future of human chromosome studies. Although these have not yet brought great dividends in terms of human welfare, I believe that the time when they will be able to do so is fast approaching.

I have tried deliberately as far as possible to confine consideration to the human chromosomes; thus on the one hand there is no discussion of possible mechanisms of cytoplasmic inheritance, or mitochondrial DNA; and on the other hand I have not included much description of work on the genetics and cytogenetics of other forms of life, animals, plants or microorganisms. Much of what is known of human genetics and cytogenetics has come through such studies; but I believe that there is now enough knowledge of human cytogenetics available to allow many problems to be tackled on the evidence from it alone, and I have preferred to leave the comparative

approach to others better qualified to deal with it, except where some topic clearly demands insertion.

The breadth of the field covered, ranging from the ultrastructure of chromosomes through the cytogenetics of human mitosis and meiosis to population cytogenetics, mutagenesis and neoplasia, and chromosome mapping, means inevitably that some topics are covered in greater depth than others. I was uncertain, for example, whether or not to include a section on molecular genetics; but decided that it would be right to do so (even if the approach will seem very elementary to most present day students) for the sake of completeness, and because of its bearing on recently introduced differential staining methods for human chromosomes, which must relate to their physicochemical structure. I have deliberately steered clear of consideration of sex determination, and the problem of intersexuality in man, partly because I have little personal experience in this complex field, and partly because Hamerton's recent book deals with such problems in considerable detail.

The reference list is somewhat like the top of an iceberg; it represents the top of a larger list, which I considered unnecessary to give *in toto*. The list given is rather loaded in favour of recent references, on the grounds that older references are more fruitfully covered by the reading of review articles. But a representative selection has been included of original references to most important topics, and these, together with the books and review articles quoted, will lead the enquiring reader as far as he or she wants to go.

It is a humbling experience to attempt to write a book of this kind; one realizes the insignificance of one's own contribution to the subject, and the immensity of one's indebtedness to the thousands who have toiled all over the world to bring our knowledge to its present state. It would be impossible to read all the relevant contributions which have been made; indeed they are probably being written even now at such a rate that no individual could read them as fast as they are produced. However, as an Englishman, I count myself extremely fortunate that English is now the most used scientific language, and I would like to express my personal gratitude to all those workers who write their papers in English, although it is not their mother tongue. If the reference list displays a certain chauvinism, this is due to the relative availability of journals to me; thus if two equally relevant papers have appeared in the *Lancet* and the *New England Journal of Medicine*, or in *Nature* and *Science, N.Y.* the former is most likely to have been selected, for the reason given above. It is in the nature of scientific research that when a new

technique is devised, it is exploited simultaneously in several different places, each of which will in due course publish comparable findings.

The last chapter, on chromosome mapping, is contributed by Dr J. H. Renwick, to whom I am extremely grateful. I was particularly anxious to include a section on this rapidly expanding subject, but I was incapable of writing an up-to-date account. Dr Renwick very kindly agreed to do this, at short notice and at considerable personal inconvenience, and I believe that his authoritative contribution, with its delightful illustration, rounds off the book in a most valuable way.

I have enjoyed writing this book, and the experience gained thereby has been invaluable. If someone, somewhere, finds it useful, I shall feel adequately rewarded.

November, 1972 E. H. R. FORD
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Acknowledgements

I should like to begin by thanking all those who have allowed me the time and afforded me the facilities to write this book. First and foremost my wife, Anna, and my family, for looking after me and helping me while I worked at home; the University of Cambridge and the Master and Fellows of Selwyn College for allowing me nine months' leave of absence; Professor R. J. Harrison (who originally suggested my writing this book) and my colleagues at the Anatomy School and Selwyn College, for arranging for my work to be done in my absence. I should also like in particular to thank my friend and collaborator, David H. M. Woollam, for the many helpful and stimulating discussions we have had together.

Special thanks are due to Raith Overhill, who drew all the original figures, and to Judith Spencer who did much of the typing; also to Ken Thurley, who gave invaluable help in the production of the electron micrographs illustrated, and to Valerie Harrison, Marie Fordham, and Pamela Mees who were severally responsible for the chromosome preparations illustrated.

I am also especially grateful to Dr Jean-Marie Luciani for allowing me to reproduce his beautiful photographs of human meiotic chromosomes, and to Dr F. R. Engmann for letting me reproduce pictures of chromosomal satellites from his Ph.D thesis.

I am also grateful to Drs C. E. Ford and H. M. Clegg for allowing me to quote extensively from their article in the *British Medical Bulletin* 25 (1965), to the late Professor L. S. Penrose and the *Lancet* for permission to reproduce Table 10, to Drs A. T. Sumner, H. J. Evans and R. A. Buckland and *Nature, New Biology* for permission to reproduce Fig. 18, and to the staff of the MRC Clinical and Population Cytogenetics Unit for supplying me with Fig. 17; also to Professor T. Caspersson and his colleagues, and the Academic Press of New York for permission to reproduce Fig. 16 (from *Experimental Cell Research*), and to Dr G. P. Clein for Fig. 15.

The material in the Appendix is quoted from the "Birth Defects Original Article Series" on the Chicago Conference, published by the National Foundation–March of Dimes, to which I express my gratitude. Finally I should like once more to thank Dr J. H. Renwick

for permission to reproduce his delightful figure of "Otto Soames" (of which he holds the copyright).

E. H. R. FORD

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CHAPTER

§ 1 §

Historical Outline

I. INTRODUCTION

The detailed study of human (and indeed of mammalian) chromosomes began in 1956. Before that year even the number was not known with certainty, and the morphology of individual chromosomes was virtually unknown. The demonstration by Tjio and Levan in 1956 that a preparation could be made which would show each individual human chromosome separately, so that the chromosomes could be accurately counted, and their shape studied, initiated a period of explosive evolution of human cytogenetics which is still continuing.

Human cytogenetics may be considered as a rather late-developing subject within the whole field of genetics. By 1956 the general principles of genetics had been firmly established for a generation at least; and the principles of cytogenetics were also well known, largely through studies on the fruit fly *Drosophila* and on plant chromosomes. These studies had started at the beginning of this century, and by the 1930s they had become highly sophisticated. The existence of this firm foundation, combined with the introduction in the last 20 years of techniques which have revolutionized the whole biological field, and which were not available when the classical studies on genetics and cytogenetics were being carried out, has enabled research carried out since 1956 to yield more information about the human chromosomes and their behaviour than is available for the chromosomes of any species other than *Drosophila* and perhaps a handful of economically important plants (Hamerton, 1971).

This book is about human chromosomes, and discussion of comparative cytogenetics has been deliberately excluded, except where it is essential to understanding. However, much of the knowledge of how human chromosomes function is based on studies on the chromosomes of other species, and particularly of plants. Hypotheses about chromosome function, which are based on the behaviour of the chromosomes of plants or of invertebrates, have been applied to explain the way in which mammalian chromosomes behave; many of them have been tested on human chromosomes, and have been found on the whole to be sound. However, the principle which has been adhered to in this book is that whenever possible the facts are drawn from experiments on mammalian and human material, and unconfirmed hypotheses based on work on other classes and phyla are treated with caution.

II. THE MAINSTREAM OF GENETICS

It is not intended here to give a critical account of the development of the whole subject of genetics; a number of competent histories of this complex subject are available. However it seems desirable to give brief consideration to the history of genetics, with two objects in mind; first, to give a framework of time within which the development of human cytogenetics may be set, and secondly to show briefly how the evolution of the subject has depended on technical advances of every kind.

The basic material of all genetics is the breeding experiment. Such experiments have been carried out from time immemorial, but were only put on a scientific basis from the seventeenth and eighteenth centuries onward. Results can not be completely assessed without the application of mathematical techniques, which were first used with any regularity by nineteenth century workers such as Francis Galton, and the immortal Gregor Mendel. Mendel's work illustrated and described the basic principles of the segregation of inherited characteristics so clearly that it has tended to overshadow that of other experimentalists of his period, even of such men as Charles Darwin.

Mendel's work was published in 1866, but it was not generally known until it was "rediscovered" by Correns, de Vries and others in 1900. Much that had occurred in the intervening period enabled it to be exploited much more rapidly than could have been possible at the

time of its publication; in particular the early development of cytogenetics.

This was a consequence of the improvement of microscopic techniques. The first description of the histology of animal tissues was made in 1827 by J. J. Lister (father of the famous surgeon), using his own compound achromatic microscope with a resolution of 1 μ. Further evolution of microscopy was itself dependent on introduction of satisfactory methods of fixation and staining of tissues, and later of section cutting. These were all introduced during the midpart of the nineteenth century, and by the last quarter of the century satisfactory methods were available. Cell division was first described by Virchow (1858), and he showed that all cells arose from other cells. In 1882 Flemming described dividing cells in the human cornea, and introduced the term "mitosis"; he also defined the framework of the nucleus as "chromatin". The term "chromosome" was introduced by Waldeyer in 1888. By that time Van Beneden had already enunciated his law that these structures were derived equally from the nuclei of two conjugating germ cells, and thus equally from each parent; and Weismann predicted the reduction division of meiosis in 1887.

Thus when the work of Mendel was rediscovered it was only a short time before it was possible to identify the segregating and recombining units of reproduction with the chromosomes, the Sutton-Boveri (Sutton, 1903) hypothesis. This is the seminal hypothesis on which cytogenetics is based. At the same time, the significance of sex chromosomes, as segregating, sex-determining structures, was realized (McClung, 1901).

Once the significance of the chromosomes had been realized (namely that they were visible subunits of the genetic material of the nucleus, in which the segregation of the genetic material could be seen to occur at meiosis), the subject of cytogenetics was vigorously pursued, particularly in two species which were especially favourable for such studies, the fruit fly *Drosophila* and maize (*Zea mays,* Indian corn). *Drosophila* was used for breeding experiments by Castle in the first decade of the twentieth century, proving suitable because of the rapidity with which it reproduced, and the large number of heritable features which could be related to the action of single genes. These features were exploited by Morgan, Bridges, Sturtevant and Muller, and enabled linkage groups on chromosomes to be identified, and chromosome maps to be made indicating the order in which the genes lay on the chromosome. In 1913 Bridges showed that the special behaviour of sex-linked genes was related to

their location on the X chromosome, and that their genetic and cytological behaviour could be directly related. Morgan exploited the cytological observation of meiotic crossing over (Janssens, 1909) to explain further the behaviour of linked genes. The whole concept of genes (a term coined by Bridges) as units of heredity strung along chromosomes like beads on a necklace was built up progressively by this group of workers, and was clearly elaborated by Morgan in his last book, "The Theory of the Gene", published in 1926. But *Drosophila* had still more to offer cytogenetics. In 1933, Painter was able to show that the banding pattern on the giant chromosomes in the salivary glands was related to the gene maps of the chromosomes, and that the bands could actually be considered as representing the linear gene sequence.

Work on maize proceeded in parallel with that on *Drosophila,* and studies on linkage were carried out in parallel with cytological studies on meiotic chromosomes. The linkage studies have been summarized by Emerson *et al.* (1935) and the cytogenetic studies by Rhoades and McClintock (1935). The meiotic chromosomes of maize show, at the pachytene stage, a variety of topographical features, particularly variation in size and distribution of "chromomeres" along their length, which enable each one to be identified individually, and cytological and genetic studies to be related as they have been in *Drosophila.* These studies added greatly to knowledge of the whole process of meiotic segregation and of the mechanism of crossing-over.

The cytological aspects of plant cytogenetics continued to be refined during the 1930s by C. D. Darlington and others; such work as was done on vertebrate chromosomes seemed to indicate that although preparations were harder to make for technical reasons, because of the smaller size and tighter packing of the animal cell as opposed to that of the plant, the general behaviour of the chromosomes both at meiosis and at mitosis paralleled that of plant chromosomes.

By the start of World War II in 1939, the general principles of genetics, and their relation to cytology, were well understood. The techniques used during this period of steady evolution of the subject were mostly already known at the beginning of the century.

III. THE GROWTH OF HUMAN CYTOGENETICS

From the time that chromosomes were recognized as significant structures, attempts were made to count the number of

chromosomes in man. However, the first significantly successful attempt was not made until 1912, when von Winiwarter counted 47 chromosomes in a spermatogonial metaphase and 23 chromosome pairs in a spermatocyte, and concluded that the human chromosome number was 48 in women and 47 in men, and that the sex-determining mechanism was the presence of either one or two X chromosomes. The next significant study was by Painter (1921, 1923) who also worked on testicular material. He observed the small Y chromosome and correctly deduced the XY sex-determining mechanism; counts of chromosomes in spermatogonial mitoses indicated that the number was between 45 and 48. In his first paper he somewhat favoured a diploid number of 46, but in his 1923 paper he decided in favour of 48, and this number was generally accepted and reported in textbooks until the work of Tjio and Levan published in 1956 clearly demonstrated that 46 was the correct number.

Although von Winiwarter adhered to his opinion that there was no Y chromosome, this was finally disproved by Koller (1937) in a study of the behaviour of the sex chromosomes during meiosis. A number of other studies of the human chromosomes were made in the interwar period, mostly on testicular material, but while these mainly supported Painter's conclusions they added little to them. Studies on mitosis in somatic cells were generally rather unenlightening, because of the technical difficulty of obtaining satisfactory preparations, and the whole subject of human cytogenetics remained rather stagnant, while the attention of geneticists was focused on the exciting discoveries which were being made on species more favourable for research. After 20 years' acceptance of a chromosome number of 48 the cautious announcement by Tjio and Levan (1956) that the human diploid chromosome number appeared to be 46 hit the cytogenetic field like a bombshell. Their counts were made on cultures of somatic cells (from fibroblasts of human embryos) and consistently gave the diploid number of 46. Moreover the quality of the preparations (illustrated in the paper) was of a different order from anything which had been seen before. The diploid number of 46 was rapidly confirmed by C. E. Ford and Hamerton (1956) on testicular material from three men, both in spermatogonial metaphases and in spermatocytes (which mostly contained 23 bivalents, or chromosome pairs).

Both these sets of observations were based on material prepared by techniques which themselves involved no materials or methods which had not been available for many years. What was new was the

combination and improvement of several techniques which had been used for different research purposes. The most important of these were the following:

(a) The use of colchicine, a substance extracted from the autumn crocus, which has been used for many years in the treatment of gout; colchicine has the specific effect of arresting cells at mitotic metaphase (by inhibiting formation of the mitotic spindle) and thus allowing many cells at mitotic metaphase to be accumulated in a culture; it has the additional advantage that since the chromosomes contract throughout mitotic prophase and metaphase, arrest at metaphase allows them to contract even more than normally, so that they can be more readily separated from each other and visualized.

(b) Pretreatment of mitotic cells with a hypotonic solution (Hughes, 1952; Hsu, 1952) which causes them to take up fluid and swell, thus facilitating the separation of the chromosomes from each other.

(c) The use of improved squash techniques, derived from techniques devised for plant cells in the 1930s; these in turn are dependent on use of the least hardening fixatives available, either acetic acid or acetic-alcohol.

The papers of Tjio and Levan and of C. E. Ford and Hamerton not only gave the correct human chromosome number; they also gave insight into techniques whereby greatly improved chromosome preparations might be made. Other workers were quick to exploit these techniques, and in two or three years' time a flood of papers on human cytogenetics began to appear which has hardly abated since. Many further technical improvements were published, particularly in relation to methods of culturing cells from various sources, and in methods of handling these cultures. These will be discussed later (Chapter 4) and only one further technical innovation will be mentioned here; this is the method of culturing lymphocytes from human blood, first described by Moorhead *et al.* (1960) which has offered a method of obtaining human chromosome preparations more easily and quickly than any other which is generally available.

IV. FACTORS INFLUENCING THE DEVELOPMENT OF HUMAN CYTOGENETICS

The growth period of human cytogenetics has come many years later than the period when the classical fundamental discoveries of