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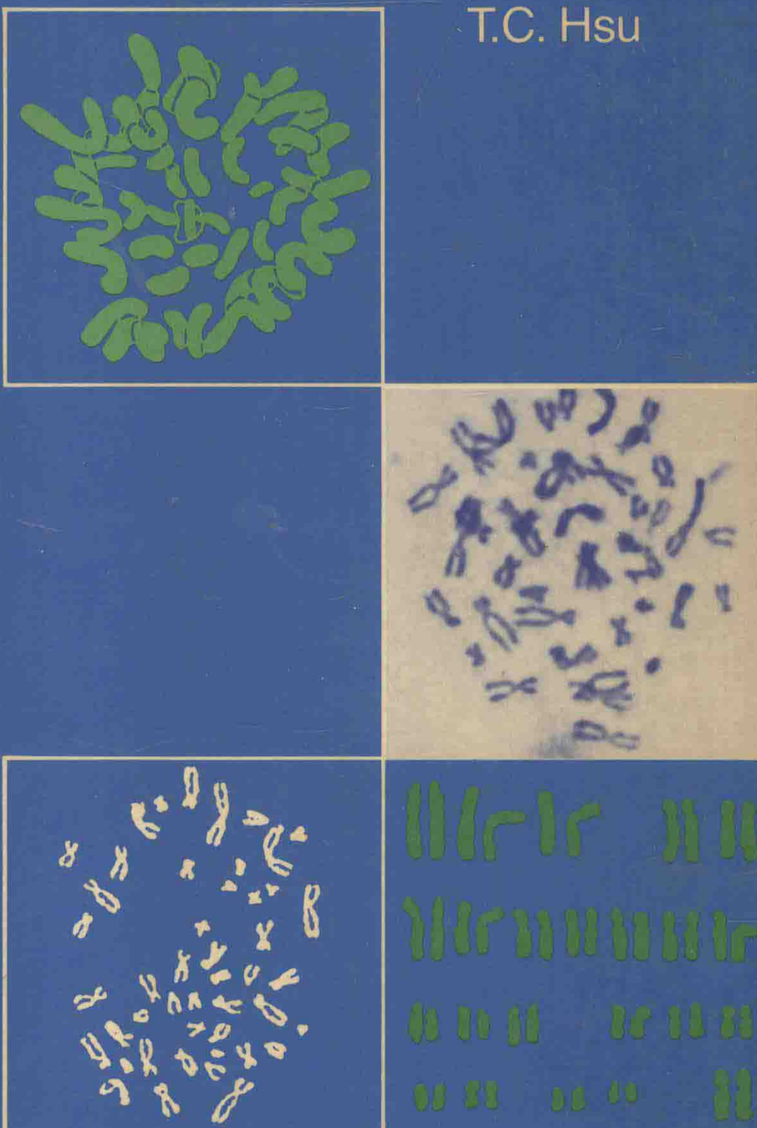
Human and Mammalian Cytogenetics

An Historical Perspective

T.C. Hsu



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With 27 illustrations

Springer-Verlag
New York
Heidelberg
Berlin

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Library of Congress Cataloging in Publication Data

Hsu, T C
Human and mammalian cytogenetics.

Bibliography: p.
Includes index.
1. Human genetics—History. 2. Mammals—Genetics—History. 3. Cytogenetics—History. I. Title.
[DNLM: 1. Cytogenetics—History. QH605 H873h]
QH431.H755 575.2'1 79-14681

Printed in the United States of America.

9 8 7 6 5 4 3 2 1

ISBN 0-387-90364-X Springer-Verlag New York

ISBN 3-540-90364-X Springer-Verlag Berlin Heidelberg

Heidelberg
Science
Library

病翼驚秋枯形閱世清得
斜陽幾度餘音更苦甚
獨抱清商頓成淒楚漫想
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一九七八夏借王船山句書懷

徐道覺

Foreword

The history of science is mostly written retrospectively, a generation or two after the actual events being discussed. Science historians are now analyzing and evaluating the origins of evolutionary and genetical theory in the nineteenth century and a sort of "Darwin industry" seems to have grown up.

A history of mammalian cytogenetics by one of the main participants is, hence, a very welcome change, since it has a vividness, an immediacy and a personal flavor which these scholarly tomes and the official biographies of scientists mostly lack. The life of the author, Chinese-born, T. C. Hsu, has been a romantic and colorful one, and he is himself a unique personality, so that his book is a very unusual blend of reminiscences, history of his special field (which has transformed human genetics) and wise comments on the mistakes made along the way.

The best qualities of a very fine Chinese mind have contributed to Dr. Hsu's career, including this book. Those qualities (which seem to me especially Chinese) include a kind of transparent honesty, a very direct empirical approach to problems and superb technical ability. And precisely these qualities seem to have been needed for success in what is likely to be seen in the future as the golden age of human and mammalian chromosome studies. The story begins with the "Dark Age," before 1956, when cytological studies of sectioned human tissues were believed to support T. S. Painter's count of 48 chromosomes. The revolution which transformed cytogenetics was the work of many cytologists in American and European laboratories. An absolutely key discovery was that treatment of living cells with hypotonic solutions would spread the chromosomes and greatly improve the quality of the preparations. A lucky accident in Dr. Hsu's laboratory (a technician misread the scale and prepared a hypotonic solution inadvertently) can be compared with the *Penicillium*-contaminated petri plate in Alexander Flemming's laboratory. In both cases a prepared and alert mind seized on an anomalous result that should not have occurred and converted it into a major discovery.

The "hypotonic miracle," combined with other technical innovations, paved the way for the discovery by Jo Hin Tjio and Albert Levan, in 1955, that the human chromosome number is, in fact, 46 and not 48. The fascinating story of the later developments, with the discovery that mongolism is due to a specific anomaly of chromosome number 21 and that numerous other human abnormalities are produced by visible alterations of the chromosomes, should be read by every biologist. This is emphatically a book which will inspire young readers: in fact I predict that quite a number of the biologists of the future will have been deflected from careers in other fields by it.

Before becoming a human (and mammalian) chromosome cytologist, Dr. Hsu was an insect geneticist. I think that his first scientific paper, published when he was still in China, was on the chromosomes of a small Chinese midge. When he came to the Texas Drosophila Laboratory in Austin early in 1948, he quickly made friends. But it was apparent to all of us that he did indeed come from some kind of bullock-wagon civilization very different from ours. The first time I took him for a trip near Austin, he saw the highway signs "U.S. 81" and concluded that the speed limit was 81 miles an hour. That was barely believable but when we turned onto U.S. 290 our new graduate student looked distinctly alarmed! However he soon overcame these initial misunderstandings and he and I made a memorable trip to collect *Drosophila* and grasshoppers all the way from Austin to Oregon. The flies collected on that trip (in spite of flash floods, predatory bears and other adventures) formed the basis for Hsu's Ph.D. dissertation. But before that achievement there were obstacles to be overcome—in particular the somewhat rigid ideas of Hsu's supervisor, the elderly Professor J. T. Patterson, who really didn't like Hsu's making any discoveries which he had not himself predicted. On the trip Hsu had discovered a new species of *Drosophila* along the Yampa River in Colorado. He asked me to suggest a name for it, so on the spur of the moment I replied that it might perhaps be called *Drosophila yampa*. Patterson bristled with indignation. Stomping down the hall he muttered to me: "That Chinese boy's full of prunes! Thinks he's got a new species—well it isn't. And he wants to call it by an *unpronounceable Chinese name*!" (Patterson later named it *Drosophila flavomontana*).

After receiving his doctorate, Hsu went to work on cultured human and mammalian cells in the laboratory of Charles Pomerat in Galveston. By this time the communist regime had taken over in China and Lysenkoism seemed to be dominant there. So Hsu remained in the United States and was eventually joined by his wife and the daughter he had never seen.

There are some biologists who work with time-honored techniques but continue to turn out new discoveries. Others are technique innovators but never discover anything but techniques. Hsu is a technique innovator who has made fundamental discoveries in cytogenetics with the aid of those techniques. It is part of his charm that his work and his book are illuminated throughout with a very special kind of warm humanity. More than any other biologist I know, T. C. Hsu seems to *care*—for his experimental animals, for his colleagues and for humanity as a whole. I think that is why this history of modern mammalian cytogenetics is so different from any other I know in the field of cytogenetics. It will surely become a classic.

M. J. D. White
Canberra, Australia

Preface

In the early 1950s, soon after I accidentally discovered the hypotonic solution pretreatment method for studying human and mammalian chromosomes, I met Professor Franz Schrader, then the coeditor of *Chromosoma*. I asked him whether *Chromosoma* would be interested in publishing a paper on the technique, which I thought had great potential. "We'll take a paper presenting good data," said he, "and you may describe your technique in the Material and Methods section."

This terse conversation exemplified the prevailing attitude at that time regarding biological research and scientific contributions: Methodological and technological achievements were subordinate to fact-finding missions and conceptual advancements. After all, a Ph.D. meant a doctor of philosophy. How could a technical device be philosophical?

Things have changed, of course. Men have gone to the moon. Biologists (imagine, biologist!) talk about cyclic AMP, nucleotide reassociation kinetics, restriction enzymes, etc., as if these subjects were born with them. People recognize that a new technique may beget new sets of pertinent facts, which in turn may beget new concepts and new theories. In this little book, I hope to portray how human and mammalian cytology, a seemingly insignificant and innocuous plaything of some microscopists who had nothing better to do, blossomed from oblivion to prominence and entered many branches of contemporary biology and medicine. And all this depended on a few technical improvements!

A number of friends urged me from time to time to write a resumé of the development of the field of human and mammalian cytogenetics. At first I hesitated because I was (and still am) not familiar with all the facets of this area of research. But then I agreed to give it a try. Since I have actually witnessed the growth of this field and have shared the excitement and frustration with fellow workers, I suppose I am one of the persons qualified to summarize the history of this branch of biology. I thought I would relate some background information on the events that led to some of the discoveries and contributions (usually only told at cocktail hours), together with brief accounts of the achievements. I thought I would also express my own opinions occasionally, right or wrong. Therefore, this book is not really very scholastic. Nevertheless, I hope it may be interesting to read. However, because of my personal involvement, it is inevitable that I shall describe more of my own experiences than those of others. To them I offer my apology. I further apologize to friends who have made contributions to this field but whose names I fail to mention.

Customarily, authors like to quote a few lines from the Bible, verses from the literature, or utterances of famous persons in the past, to decorate their books or chapters. I choose to quote a few verses from an old Chinese poem as my frontispiece. The poem was composed in the late Sung Dynasty by Wang Yi-sun. Ostensibly, it describes a cicada who, singing in sobbing notes against the autumn winds, yearns for the sweet summer breeze sweeping through willow branches and bemoans the anticipation that he has not many sundowns to go. Of course the poet metaphorically lamented over his own impending age and his lack of fulfillment. It is not exactly a delight for a nonhistorian to write an historic account of a subject in which he played a role, for this only suggests that he, too, yearns for the sweet summer breeze!

Acknowledgments

In the course of preparing this book, many friends lent assistance in a variety of ways. Some provided me with information, others gave illustrative materials, and still others offered encouragement, criticisms, and comments on the draft. I would like to give my sincerest thanks to these: Drs. Ulfur Arnason, Frances E. Arrighi, Murray L. Barr, Hans Bauer, Kurt Benirschke, Larry L. Deaven, A. C. Fabergé, Patricia Farnes, Joseph G. Gall, David A. Hungerford, Harold P. Klinger, Jérôme

Lejeune, Albert Levan, C. C. Lin, Dan Lindsley, Sajiro Makino, Robert Matthey, Orlando J. Miller, Peter C. Nowell, Joseph T. Painter, Mary Lou Pardue, Sen Pathak, Rudolph Pfeiffer, T. T. Puck, Potu N. Rao, Margery W. Shaw, Elton Stubblefield, Jo Hin Tjio, and Jacqueline Wang-Peng. I also would like to thank my secretaries who typed and retyped the manuscript as I changed my mind: Mlles. Mary Bea Cline, Dorothy Holitzke, and Patricia Hall. A special gratitude is extended to Miss Joan McCay, who practically twisted my arms in urging me to write this book and who got me over my inertia.

T. C. Hsu

Contents

1 Introduction

The science of human and mammalian cytogenetics began very slowly. During the last two decades, however, it has made some remarkable progress, and increased activity shows no sign of abatement. For example, the arena for investigations on chromosomes has traditionally been the research laboratories, which tackled a variety of problems of fundamental importance. But today, investigations are also carried out in medical institutions as routine tools for diagnosis, prognosis, and counseling.

Probably few cytogeneticists would disagree that the history of human and mammalian cytogenetics can be divided roughly into four periods, separated by some significant events. The first period can be called the prehypotonic era, in which the use of classic sectioned or squash preparations of tissues *in situ* was the prevailing practice. I have written only a single chapter (Chapter 2) to review the activities of that era, since most of the data and conclusions of the period were later found to be erroneous or in need of revision.

The second period (Chapters 3–5) lasted seven years (1952–1959), including the rediscovery of the hypotonic solution pretreatment for cytological preparations, which turned out to be a key to the development of the field of human and mammalian cytogenetics. During this period, the diploid number of man, long believed to be 48, was corrected to 46. Characterization of the human chromosomes also began. In addition, some approaches developed by cell biologists, such as autoradiography and cell cloning, began to be utilized to study chromosomes.

Still, the activities during this period were minimal compared to those of the next two periods, which were stimulated by a number of factors—especially the discovery of trisomy (one or more triploid chromosomes) in man.

The third period (1959–1969) saw explosive activities in human cytogenetics and the beginning of research into mammalian chromosomes (Chapters 6–15). Needless to say, the finding that a clinical syndrome is intimately associated with an abnormal chromosomal constitution stimulated the progress of medical cytogenetics. In the meantime, the somatic cell hybridization system was established as a new tool to correlate genetics and cytology in higher animals.

The modern period (1969–onward) started with the invention of techniques to characterize individual metaphase chromosomes and to subdivide each chromosome into visually recognizable zones or bands. Without such techniques, much of the earlier work would have remained ambiguous or even erroneous. Biologists also began to combine molecular biology and cytology, and a new field, molecular cytogenetics, emerged. A cursory inspection of cytogenetic journals such as *Chromosoma* shows a heavy influx of articles in recent years dealing with molecular biology or molecular cytogenetics, whereas 10 or 15 years ago few could be found. Chapters 16–23 describe some of the events of this period.

The divisions of the history of human and mammalian cytogenetics into periods are marked by some landmark events and the dominant research activities that followed. This does not mean that research of the previous period ceased when a new period arrived. Nor does it mean that one period of research did not benefit from the research of the past. However, for convenience in describing a subject, I have chosen to present each subject in one chapter to avoid duplication. For example, somatic cell hybridization started early in the 1960s, and many contributions were made in that decade. But I present the subject in the modern period to avoid presenting it in two chapters.

The last chapter represents my personal views on what might come in the future. Naturally, these are all speculation, and the actual cause of development may be completely different.

It would be impractical to cite a large number of references because the bibliography would be enormous. However, I have cited some pertinent references mentioned in the text and have added a few review papers and books that are not referred to in the text but are excellent reading materials. References in the latter category are gathered as a list of suggested readings that precedes the cited references.

2 The Knights of the Dark Age

A few years ago, I was invited to participate in the Symposium on Biological Clocks, organized by the Argonne National Laboratory. Often before, my presentations had been arranged (logically) to follow papers on biochemistry and molecular biology, and that symposium was no exception. By way of an introduction, I commented that, with the presentations on biochemistry and molecular biology behind us, we were leaving the precise studies and entering the realm of ambiguity. I also commented that whereas molecular biology is beautiful—because of the imaginative ideas behind the research, the neat experimental protocol, the precise measurements of the results, and the meaningful interpretations—the beauty is primarily abstract, similar to that of many abstract paintings. No one gets much visual pleasure from a scintillation plot or a density gradient tracing. On the other hand, microscopic objects, particularly chromosomes, are themselves objects of beauty, analogous to the beauty of a Rembrandt.

My topic of that meeting was the kinetochore structure and the relationships between the kinetochore and the spindle microtubules, a collaborative work with my former colleague Bill Brinkley. I supplemented my presentation with many electron micrographs. Herbert Stern, the speaker after me, said, "Dr. Hsu compared his electron micrographs with Rembrandt paintings for visual enjoyment. I guess his electron micrographs and Rembrandt paintings have something in common: Lots of shade and not much light." He then proceeded to tell the audience that his Feulgen-fast green photos of plant chromosomes were more like Renoirs.

I tell this story of friendly jest to illustrate that chromosomes have attracted many microscopists not only because these sausage-like bodies represent vehicles of genetic material (and, hence, are biologically important) but also because they are hypnotically beautiful objects. Sometimes, after having obtained the information they sought, microscopists would continue to examine cytological preparations just for the enjoyment of looking at more mitotic or meiotic figures.

In the first half of this century, the development of cytogenetics relied heavily on studies of plant and insect chromosomes. Many important discoveries were made during examinations of these materials, and techniques were standardized. Yet the chromosome cytology of one of the most important groups of life forms, the vertebrates, was disproportionately underdeveloped. It is clear now that the great majority of data on vertebrate cytogenetics derived during this period were unreliable, and it is no exaggeration to call the first half of this century the Dark Age of mammalian and human cytogenetics.

During this period, there were two important technical improvements in chromosome cytology—the squash preparation methods and the colchicine pretreatment method—both invented by plant cytologists. Before Belling (1921) introduced the squash technique, cytologists used the paraffin section method to obtain their preparations. For studies of tissue organization (histology, embryology, and pathology), this traditional method is indispensible even today. For cytological observations, however, the method has several disadvantages, among which is the likelihood of cutting a cell into several slices. A long chromosome might be cut into two or three segments, and, even though serial sections can be examined, errors are unavoidable.

Squash preparations eliminate all the defects inherent in sectioned preparations. The intactness of the cell is preserved, allowing no doubt as to the completeness of the chromosome complement or the individual chromosomes. Moreover, the pressure applied during squashing (by thumb or sometimes even by tapping or pounding) forces the cells to flatten, causing all chromosomes to lie on one plane of focus. Frequently, the pressure forces the