Benchmark Papers
in Genetics V. 14

SOMATIC CELL GENETICS

Edited by

Richard L. Davidson

Hutchinson Ross Publishing Company

Benchmark Papers in Genetics / 14

A BENCHMARK® Books Series

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Stroudsburg, Pennsylvania

Benchmark Papers in Genetics, Volume 14
Library of Congress Catalog Card Number: 83-10810
ISBN: 0-87933-120-8

86 85 84 1 2 3 4 5
Manufactured in the United States of America.

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA
Main entry under title:
Somatic cell genetics.
(Benchmark papers in genetics; 14)
Includes indexes.
1. Somatic hybrids—Addresses, essays, lectures.
2. Cytogenetics—Addresses, essays, lectures.
I. Davidson, Richard L. II. Series. [DNLM: 1. Hybrid cells—Collected works. 2. Hybridization—Collected works. 3. Mammals—Collected works. 4. Mutation—Collected works. 5. Transformation, Genetic—Collected works. WL BW516 v.14 / QH 425 S693]
QH451.S64 1984 599'.0873223 83-10810
ISBN 0-87933-120-8

Distributed worldwide by Van Nostrand Reinhold Company Inc.,
135 W. 50th Street, New York, NY 10020.

SERIES EDITOR'S FOREWORD

The study of any discipline assumes mastery of the literature of the subject. In many branches of science, even one as new as genetics, the expansion of knowledge has been so rapid that there is little hope of learning of the development of all phases of the subject. The student has difficulty mastering the textbook, the young scholar must tend to the literature near his own research, the young instructor barely finds time to expand his horizons to meet his class preparation requirements, the monographer copes with a wider literature but usually from a specialized viewpoint, and the textbook author is forced to cover much the same materials as previous and competing texts to respond to the user's needs and abilities.

Few publishers have the dedication to scholarship needed to serve the limited market of advanced studies. The opportunity to assist professionals at all stages of their careers has been recognized by the publishers of the Benchmark series and by a distinguished group of Benchmark volume editors knowledgeable in specific aspects of the genetics literature. Some have contributed greatly to the development of that literature, some have studied with the early scholars, and some have developed and are in the process of developing entirely new fields of genetic knowledge. In many cases the judgments of the editors become a historical document that records their opinion of the important steps in the development of the subject. The editor of this volume has selected papers and portions of papers that demonstrate both the development of knowledge and the atmosphere in which that knowledge was developed. There is no substitute for reading great papers. Here you can learn how questions are asked, how they are approached, and how difficult and essential it is to obtain definitive answers and clear writing.

Dr. Davidson traces the development of somatic cell genetics of mammalian cell systems considering papers in somatic cell hybridization, gene transfer, and mutant cells. These papers emphasize the importance of the combined approach of cell genetics and recombinant DNA to approach questions of development, regulation, and growth of normal and tumor cells. Gene mapping and gene transfer are also considered. *Somatic Cell Genetics* represents genetics as modern molecular biology, and as such is a welcome addition to the series.

DAVID L. JAMESON

PREFACE

Somatic cell genetics is a relatively young field of study; its origins date back little more than two decades. During its development somatic cell genetics drew heavily on the techniques and concepts of many other fields such as microbial genetics, biochemistry, and molecular biology. As it matured, however, the field of somatic cell genetics introduced a variety of important techniques and concepts of its own. Somatic cell genetics has provided major new approaches to the study of gene regulation and genetic organization in mammalian cells, and has made possible the manipulation of the mammalian genome in vitro, with the movement of genes even across species barriers. The techniques of somatic cell genetics have now become a standard feature of research in many diverse areas, ranging from the isolation of genes to the production of immunological reagents.

In this book I have attempted to trace the major developments in somatic cell genetics by presenting 47 classic papers. For the sake of clarity, these papers are grouped into three major areas—somatic cell hybridization, gene transfer, and mutant cells. Clearly, many more than 47 papers could be used to trace the development of somatic cell genetics. However, because of space limitations, many important papers could only be cited in the commentaries. Furthermore, in several cases, significant advances were reported simultaneously by different laboratories. Since it was possible to present only one of these papers in each case, arbitrary choices had to be made. However, I have attempted to acknowledge the contributions of the laboratories whose work could not be published in this volume. In considering papers on mutant cells, I found it necessary to establish a specific focus because the range of mutant cell studies has expanded in so many directions. My choice of papers on this topic was influenced by the relevance of the results for studies on somatic cell hybridization and gene transfer. This criterion provided a somewhat objective mechanism for choosing between papers when comparable principles were established with different mutant cell systems.

A number of important topics were intentionally not considered in order to provide a more focused presentation. Among the topics avoided were cytogenetics, X chromosome inactivation, and cell membranes. Finally, in choosing papers for this book, I have focused on mammalian cell systems. This focus reflects not only my own personal interests, but also the predominant role that mammalian systems have played in the development of somatic cell genetics.

The major problem, and also the most interesting aspect of making a compilation of papers, as in this volume, is the determination of what is a classic paper. To facilitate this determination I have tried to follow certain criteria. Clearly, papers that introduce important new techniques or establish new principles should be considered as classics. Other types of papers also may be considered as classics because of their impact on subsequent research. For example, some papers are included because they raise important questions and have stimulated much activity in other laboratories. I did not consider it necessary that these papers provide the eventual solution; raising the question is sufficient. I am also aware that the significance of some of the older papers reprinted in this volume is no longer widely acknowledged. However, such opinion does not alter the historical role of these papers since their influence, even if not currently recognized, is reflected in the present status of somatic cell genetics.

As already mentioned, one measure of a classic paper is its impact on subsequent research. By definition, therefore, it is much easier to recognize an old classic than a new one. Nevertheless, a number of recent papers have been included. These papers, for example, in the area of gene transfer, were chosen because I expect them to establish trends for the future. More time must pass before the validity of these selections can be assessed.

I would like to thank my wife Jalane for much help in writing this book. I also thank Dr. Raju Kucherlapati for critically reading the manuscript and Linda Cardenas for her help in preparing the manuscript.

RICHARD L. DAVIDSON

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INTRODUCTION

Somatic cell geneticists have nothing against sex. However, sexual reproduction is not well suited for the genetic analysis of mammals and it is especially unsuited for the genetic analysis of man. All of the prerequisites for stringent genetic studies such as controlled matings, short generation times, and large numbers of progeny, are largely or completely missing in mammalian reproductive systems. In the last few decades, therefore, many scientists interested in mammalian genetics have turned their efforts to genetic systems that bypass sexual reproduction, that is, to what we now call *somatic cell genetics*.

In somatic cell genetics, somatic cells rather than germ cells are the basis for genetic analysis. With mammalian somatic cells growing in culture almost like microorganisms, the techniques of mutant selection, cell hybridization, and gene transfer can be used to introduce genetic variation into cells and to mix genomes in new combinations. Through the use of somatic cells it is possible to satisfy the prerequisites for high-resolution genetic analysis: genomes can be combined at will, without even respecting species barriers; the generation times of cultured cells are relatively short, about one day for a human cell in culture compared to more than twenty years for a human being; and large numbers of progeny from independent genetic events can be obtained.

By using the techniques of somatic cell genetics a variety of genetic questions that are difficult to approach via sexual reproduction are amenable to experimental analysis with cultured mammalian cells. These questions relate to gene regulation, genetic variation, and the formal genetics (chromosome mapping) of man.

Somatic cell hybridization was discovered in 1960 as an unexpected result of experiments on the cocultivation of mammalian cells of different types. Early studies on somatic cell hybridization focused on the types of cells that could be hybridized, on methods for isolating hybrid cells, and on techniques for increasing the frequency of cell hybridization. In 1964 the "HAT" selective system

was introduced as the first system for the selection of somatic cell hybrids. With this selective system it became possible to isolate pure populations of hybrid cells that occurred at only very low frequencies. Soon thereafter, it was found that viable hybrids could even result from fusion between cells of different species or different tissues. The availability of selective techniques and the ability to produce hybrids between cells of different species or tissues led to a rapid expansion of activity in the area of somatic cell hybridization.

Following the early descriptive and methodological studies on somatic cell hybridization, work on cell hybrids focused on two main areas: gene regulation and gene mapping. Somatic cell hybridization made it possible to combine within a single nucleus the genomes of cells that differed in their patterns of gene expression, and this provided new opportunities for analyzing the mechanisms of genetic regulation in mammalian cells. The existence of interspecific hybrid cells, which in certain cases segregated chromosomes of one of the parental species, allowed gene mapping studies to be performed with cultured cells. Somatic cell hybridization has proven to be the most productive way of mapping the human genome yet developed. More recently, the techniques of cell hybridization have been used to construct cells that synthesize *monoclonal* antibodies. These highly specific antibodies appear to have significant potential as immunological reagents for medical as well as scientific purposes.

Work on gene transfer began at about the same time as the early work on somatic cell hybridization. The first apparently successful transfer of a genetic trait from one mammalian cell to another was reported in 1962 and involved cellular DNA as the transforming vector. However, in contrast to the rapid progress of work on cell hybridization, work on gene transfer advanced slowly after the initial report, and it was not until a decade later that reproducible gene transfer systems for mammalian cells were developed. In 1971 a system was introduced in which Herpes simplex virus, rather than cellular DNA, was the transforming vector. This viral transformation system provided the basis for much of the gene transfer work accomplished to date, and it led to the eventual development of reliable and efficient systems for transformation by means of mammalian cell DNA.

With recent developments in recombinant DNA technology permitting the isolation of specific genes, work on gene transfer systems has become increasingly active. The introduction of foreign genes into cells, and more recently into embryos, should play a major role in attempts to elucidate the molecular mechanisms of gene regulation in higher organisms.

Work on mutant cells began well before the work on cell hybridization and gene transfer. Clearly, studies on the inheritance of variation go back to the very origins of the study of genetics. However, studies on mutant mammalian cells date back only three decades. The initial studies in this area were carried out not for the purpose of characterizing mutant mammalian cells per se, but rather to study the mechanisms by which tumors developed resistance to antitumor drugs. In 1950 the development of leukemic lines resistant to the folic acid antagonist amethopterin was reported, and the following year leukemic lines resistant to the purine analog 8-azaguanine were described. As will be discussed later, studies involving these two drugs have had a major impact on the field of somatic cell genetics. In fact, a history of somatic cell genetics could be largely centered around these two drugs. The "HAT" selective system, which was developed as a result of studies with these drugs, has made possible much of the work on cell hybridization and gene transfer accomplished to date. Furthermore, studies on the drug-resistant cells themselves have provided much of our current information on the alterations in mutant mammalian cells.

During the mid-1950s, techniques for cloning mammalian cells in culture were being developed and improved. These techniques, which permitted the isolation of pure lines of genetically marked cells, were essential to the development of somatic cell genetics. (For discussion of these techniques the reader should refer to general texts on cell culture.) In addition, the improved definition of the nutritional requirements of cultured mammalian cells quickly led to the isolation and characterization of nutritional variants of cultured cells. In 1960 the first method for isolating auxotrophic mutants of mammalian cells was described. Conditional lethal mutants, such as auxotrophic and temperature sensitive mutants, have provided much information on the physiology and genetics of cultured mammalian cells.

In all areas of somatic cell genetics, the techniques of cell genetics and of recombinant DNA are being jointly applied, and this combined approach is becoming increasingly more powerful and productive. Major developments can be anticipated in the various areas in which the techniques of somatic cell genetics and recombinant DNA have been joined, including gene regulation, gene mapping, embryology, and tumor biology.