# MAMMALIAN CELL GENETICS

MARTIN L. HOOPER

# MAMMALIAN CELL GENETICS

MARTIN L. HOOPER
*Department of Pathology*
*University Medical School*
*Edinburgh, United Kingdom*

A WILEY-INTERSCIENCE PUBLICATION
JOHN WILEY & SONS
New York • Chichester • Brisbane • Toronto • Singapore

***Library of Congress Cataloging in Publication Data:***

Hooper, Martin L.
Mammalian cell genetics.

(Cell biology ; v. 4)
"A Wiley-Interscience publication."
Includes bibliographical references and index.
1. Mammals—Genetics. 2. Cytogenetics. I. Title.
II. Series: Cell biology (New York, N.Y.) ; v. 4.

QL738.5.H66 1985 599'.0873223 84-17425
ISBN 0-471-89201-7

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

# MAMMALIAN CELL GENETICS

## CELL BIOLOGY: A SERIES OF MONOGRAPHS

*E. Edward Bittar, Series Editor*

---

**Volume 1: PHOTOSYNTHESIS**
*Christine H. Foyer*

**Volume 2: CELL AND TISSUE REGENERATION: A BIOCHEMICAL APPROACH**
*Margery G. Ord and Lloyd A. Stocken*

**Volume 3: PLANT MEMBRANES: ENDO- AND PLASMA MEMBRANES**
*David G. Robinson*

**Volume 4: MAMMALIAN CELL GENETICS**
*Martin L. Hooper*

# SERIES PREFACE

The aim of the Cell Biology Series is to focus attention upon basic problems and show that cell biology as a discipline is gradually maturing. In its largest aim, each monograph seeks to be readable and informative, scholarly, and the work of a single mind. In general, the topics chosen deal with major contemporary issues. Together they represent a rather large domain whose importance has grown enormously in the course of the last generation. The introduction of new techniques has no doubt ushered in a small revolution in cell biology. However, we still know very little about the cell as an ordered structure. As will become abundantly clear to the reader, real progress is not just a matter of progress of technique but also a matter of close interaction between advances in different fields of study, as well as genesis of new approaches and generalized concepts.

E. EDWARD BITTAR

*Madison, Wisconsin*
*January 1984*

# PREFACE

This book is concerned with the genetic analysis of cells taken from a multicellular mammal and cultured as though they were independent microorganisms. This form of analysis has come to be known as somatic cell genetics, although its application is not restricted to somatic cells. My aim was not to write a textbook. The student requiring an introduction to somatic cell genetics is already well served by the books of Puck (1) and Littlefield (2) which describe the early development of the subject, and by the recent, more comprehensive texts of Morrow (3) and Martin et al. (4). Rather, I have tried to bridge the gap between such works and multiauthor volumes such as those edited by Caskey and Robbins (5) and Shay (6) which describe selected aspects of the subject in depth. This book is aimed at research workers who either are about to enter the field of mammalian somatic cell genetics, or have experience in one area of the field but require a guide to the literature in other parts of it. To keep the size of the book within manageable bounds, I have set myself the limited aim of making the reader aware of work described elsewhere rather than of explaining it in detail. In other words, the book is designed not to stand alone but to be read in conjunction with the primary literature. This explains the lack of explanatory diagrams, which would be a serious omission in a more introductory text, and the relative profusion of tables.

Somatic cell genetics has close relationships both with microbial genetics, from which it largely derives its strategies and approaches, and with the classical genetics of higher eukaryotes, with which it shares its aims. For the genetic analysis of mammals the somatic cell genetic approach has certain advantages compared to the classical approach: The environment of the individual cell is better controlled in culture, and the generation time of the cell is much shorter than that of the whole organism. It also has drawbacks: First, the very fact that the culture environment differs from a cell's environment in the whole organism imposes selection in favor of abnormal cells; second, cells *in vivo* interact with other cells of the same organism both through direct cell contact and via diffusible signal molecules. Some such interactions occur in cell culture, limiting the extent to which cells behave as independent microorganisms. Other interactions do not occur in cell culture, limiting the range of phenomena that can be studied. Nevertheless, because the techniques available to the somatic cell geneticist and the classical geneticist are different, the kinds of information available from the two approaches are often complementary, and systems where they can be combined are particularly powerful (see Chapter 6).

The text is organized in terms of techniques and approaches rather than in terms of applications. Access to a particular application may be made from the Index. Chapter 1 gives an overview of the basic techniques of mammalian cell culture, although some knowledge in this area is assumed and emphasis is placed upon those aspects of direct relevance to later chapters. Chapter 2 discusses selective techniques, including some which have not yet been extensively applied in somatic cell genetics but have potential for future work. Chapters 3 and 4 deal with the central topics of cell variants and cell hybrids. Chapter 5 is concerned with a major growth area, namely, the application of the techniques of molecular genetics to cultured cells. The inclusion of Chapter 6 is partly a reflection of my own interest in teratocarcinomas but mainly a consequence of the potential of work in this area for unifying the genetics of the cell with that of the organism. I have sought to emphasize the relationships between the different branches of the subject, with the more classical work described in the first four chapters providing a link between the molecule and the multicellular organism.

For the convenience of the reader I have not given references to the primary literature in all cases but have often cited reviews from which the original references may obtained. I hope that authors who, for this reason, have not been acknowledged will forgive me.

There is as yet no universally accepted system of abbrevations in use in somatic cell genetics. The one I have adopted is consistent with the majority of current usage: Gene products are given capital letters, for example, TK (thymidine kinase); variant phenotypes are also given in capitals, for example, $TK^-$ (thymidine kinase defective) and $OUA^R$ (ouabain-resistant); gene loci are given in lower case italic letters, for example, *tk*; complementation groups are given with an initial capital letter, for example, UrdA.

I am grateful to many colleagues for valuable discussions that have shaped this book. The idea of writing it can be traced back to a conversation with Ben Carritt some years ago. Professor Sir Alastair Currie and Austin Smith kindly read the manuscript and made a number of constructive suggestions. Kit Gardner provided expert secretarial assistance. The shortcomings, which may remain, are entirely my own responsibility and I hope that readers will be kind enough to point them out to me.

MARTIN L. HOOPER

*Edinburgh, United Kingdom*
*October 1984*

# CONTENTS

# 1

# THE CULTURED MAMMALIAN CELL

The methods of mammalian cell genetics have been developed by combining strategies largely derived from microbial genetics with the techniques of mammalian cell culture. It is assumed that the reader is familiar with basic mammalian cell culture techniques, therefore only a broad overview is given here to put into context those aspects of particular relevance to later chapters. More detailed information will be found in references 7–11. The composition of growth media (Section 1.1) forms the basis of the majority of the selective systems described in Chapter 2. The requirement of most cell types for attachment to a substratum (Section 1.2) also constrains the design of selective systems, particularly in selecting for cells present at very low frequencies in a population where a large input of cells, and therefore a large surface area, is required. Subculturing (Section 1.4), cloning (Section 1.9), and cryopreservation (Section 1.10) of selected cells are also of paramount technical importance. The remainder of the chapter deals with the relationship between tissue-culture cells and cells in normal tissues. Most of the techniques of mammalian cell genetics cannot be carried out directly on primary cultures (Section 1.3) but require the establishment of permanent lines (Section 1.5). The relationship of cells of such lines to normal cells in chromosome constitution, state of differentiation, and interaction with other cells is discussed in Sections 1.6, 1.7, and 1.8. To date, the majority of work in somatic cell genetics has been with "fibroblast-like" cells (Section 1.5), but cell lines are now available that exhibit different states of differentiation and are suitable for use in somatic cell genetics (Section 1.7).

## 1.1. GROWTH MEDIA

Early tissue-culture experiments used biological fluids such as serum as growth media. As biochemical characterization of serum components proceeded, numerous formulations of chemically defined media were developed for particular cell types (reviewed by Waymouth, ref. 12). Although some of these were designed to reduce the requirement for serum protein to minimal levels none is adequate for completely serum-free growth of any cell line with the exception of certain lines that have undergone strong selection for ability to grow *in vitro* and are as a consequence highly aneuploid (Sections 1.5 and 1.6). For this reason relatively simple media have become popular, such as Eagle's minimum essential medium (MEM, Table 1.1) which was

designed to reduce the requirement for amino acids and vitamins to the minimum necessary for use with a supplement of 5–10% serum. Nonetheless, considerable effort has been devoted to defining all the low-molecular-weight growth-promoting molecules contributed by serum and this has led to the development of media such as Ham's F12 and more recently MDCB105 (Table 1.1) which are sufficiently complete to allow the serum requirement to be fulfilled for certain cell types by purified hormones or macromolecular serum fractions (Section 1.1.9). The closer one approaches a completely defined medium, the more marked become the differences in the requirement of different cell types, which emphasizes the point that the growth of one cell type in medium whose composition has been optimized for another, which is a widespread practice in tissue-culture laboratories, may impose a considerable selective pressure in favor of abnormal cells.

The majority of normal cell types show anchorage-dependent growth, although cell lines capable of growth in suspension can be obtained from normal and malignant hematopoietic cells (14) and by adaptation of monolayer cell lines such as HeLa and L cells (7). Media for suspension cultures are very similar to those used for monolayer culture, the principal modifications being a lowering of the $Ca^{2+}$ ion concentration, the use of somewhat higher concentrations of nutrients and serum, and the incorporation of carboxymethyl cellulose to overcome precipitation problems at the gas/liquid interface (7,15).

### 1.1.1. Carbon Source

The most commonly used carbon source in cell culture media is glucose, although many other sugars can be used instead, and galactose has the advantage that its metabolites lead to less acidification of the medium than do those of glucose (12,19). In addition, sodium pyruvate is commonly added to media designed for use at low cell density as unlike the phosphorylated intermediates of glycolysis it is readily lost from the cytoplasm to the external medium if the concentration gradient across the cell membrane is high.

### 1.1.2. Major Salts, Buffers, and the Gas Phase

The principal constraints determining the concentrations of major inorganic ions in tissue-culture media are total osmolarity and ionic balance (19).

Media are generally designed to be isoosmotic with serum, although deviations of up to 10% from the nominal osmolarity appear to be well tolerated, at least by L cells. Na/K ratios appear to be particularly important, and different ratios are optimal for different cell lines. For pH maintenance bicarbonate buffers requiring equilibration with an atmosphere of 5–10% $CO_2$ are most commonly employed. Use of other buffers is complicated by the fact that cells appear to have a metabolic requirement for $CO_2$ in addition to a requirement for pH maintenance, and also that tris and phosphate buffers used alone exhibit some toxicity at concentrations required to ensure adequate buffering. In order to dispense with the need for gassing with $CO_2$/air, Leibovitz (20) employed phosphate and high concentrations of arginine and histidine to achieve buffering, together with pyruvate to generate endogenous $CO_2$ and galactose as a carbon source to reduce the production of lactic acid. Other successful strategies include the use of β-glycerophosphate or HEPES to provide major buffering capacity with the addition of a low concentration of bicarbonate (19,21,22).

Oxygen tension is also an important variable (19,22). Although 5% $CO_2$/95% air is most commonly used for gassing cell cultures there are indications that the optimal oxygen concentration in the gas phase may be as low as 1–3% (23), and hyperbaric oxygen levels such as are helpful for maintenance of tissue fragments are markedly toxic to cell cultures (19,22). This toxicity is most severe under conditions of selenium deficiency (see Section 1.1.6). Vitamin E may exert a protective effect (24).

### 1.1.3. Amino Acids

In general ten amino acids are essential in the mammalian diet (Table 1.1) and these are included in tissue-culture media together with cysteine (or cystine), glutamine, and tyrosine, whose synthesis is restricted to certain tissues only and which are therefore essential for the majority of cell types. In addition media designed for use at clonal cell density include the remaining seven amino acids of the twenty which are protein constituents. These can be synthesized by cells but are readily lost to the medium if the transmembrane concentration gradient is high (compare pyruvate, Section 1.1.1).

### 1.1.4. Vitamins

Whereas the B vitamins (Table 1.1) are essential for the growth of cultured cells, the fat-soluble vitamins appear not to be (24). Ascorbate may have