

# Methods in Cell Biology

Edited by DAVID M. PRESCOTT

VOLUME VI

# Methods in Cell Biology

*Edited by*

DAVID M. PRESCOTT

DEPARTMENT OF MOLECULAR, CELLULAR AND  
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VOLUME VI

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## PREFACE

In the ten years since the inception of the multivolume series *Methods in Cell Physiology*, research on the cell has expanded and added major new directions. In contemporary research, analyses of cell structure and function commonly require polytechnic approaches involving methodologies of biochemistry, genetics, cytology, biophysics, as well as physiology. The range of techniques and methods in cell research has expanded steadily, and now the title *Methods in Cell Physiology* no longer seems adequate or accurate. For this reason the series of volumes known as *Methods in Cell Physiology* will now continue under the title *Methods in Cell Biology*.

Volume VI of this series continues to present techniques and methods in cell research that have not been published or have been published in sources that are not readily available. Much of the information on experimental techniques in modern cell biology is scattered in a fragmentary fashion throughout the research literature. In addition, the general practice of condensing to the most abbreviated form materials and methods sections of journal articles has led to descriptions that are frequently inadequate guides to techniques. The aim of this volume is to bring together into one compilation complete and detailed treatment of a number of widely useful techniques which have not been published in full detail elsewhere in the literature.

In the absence of firsthand personal instruction, researchers are often reluctant to adopt new techniques. This hesitancy probably stems chiefly from the fact that descriptions in the literature do not contain sufficient detail concerning methodology; in addition, the information given may not be sufficient to estimate the difficulties or practicality of the technique or to judge whether the method can actually provide a suitable solution to the problem under consideration. The presentations in this volume are designed to overcome these drawbacks. They are comprehensive to the extent that they may serve not only as a practical introduction to experimental procedures but also to provide, to some extent, an evaluation of the limitations, potentialities, and current applications of the methods. Only those theoretical considerations needed for proper use of the method are included.

Finally, special emphasis has been placed on inclusion of much reference material in order to guide readers to early and current pertinent literature.

DAVID M. PRESCOTT

# CONTENTS OF PREVIOUS VOLUMES

## *Volume I*

1. SURVEY OF CYTOCHEMISTRY  
R. C. von Borstel
2. METHODS OF CULTURE FOR PLASMODIAL MYXOMYCETES  
John W. Daniel and Helen H. Baldwin
3. MITOTIC SYNCHRONY IN THE PLASMODIA OF *Physarum polycephalum*  
AND MITOTIC SYNCHRONIZATION BY COALESCENCE OF  
MICROPLASMODIA  
Edmund Guttes and Sophie Guttes
4. INTRODUCTION OF SYNCHRONOUS ENCYSTMENT (DIFFERENTIATION) IN  
*Acanthamoeba* sp.  
R. J. Neff, S. A. Ray, W. F. Benton, and M. Wilborn
5. EXPERIMENTAL PROCEDURES AND CULTURAL METHODS FOR *Euplotes*  
*eurystomus* AND *Amoeba proteus*  
D. M. Prescott and R. F. Carrier
6. NUCLEAR TRANSPLANTATION IN AMEBA  
Lester Goldstein
7. EXPERIMENTAL TECHNIQUES WITH CILIATES  
Vance Tartar
8. METHODS FOR USING *Tetrahymena* IN STUDIES OF THE NORMAL CELL  
CYCLE  
G. E. Stone and I. L. Cameron
9. CONTINUOUS SYNCHRONOUS CULTURES OF PROTOZOA  
G. M. Padilla and T. W. James
10. HANDLING AND CULTURING OF *Chlorella*  
Adolf Kuhl and Harald Lorenzen
11. CULTURING AND EXPERIMENTAL MANIPULATION OF *Acetabularia*  
Konrad Keck
12. HANDLING OF ROOT TIPS  
Sheldon Wolff
13. GRASSHOPPER NEUROBLAST TECHNIQUES  
J. Gordon Carlson and Mary Esther Gaulden

14. MEASUREMENT OF MATERIAL UPTAKE BY CELLS: PINOCYTOSIS  
Cicily Chapman-Andresen
15. QUANTITATIVE AUTORADIOGRAPHY  
Robert P. Perry
16. HIGH-RESOLUTION AUTORADIOGRAPHY  
Lucien G. Caro
17. AUTORADIOGRAPHY WITH LIQUID EMULSION  
D. M. Prescott
18. AUTORADIOGRAPHY OF WATER-SOLUBLE MATERIALS  
O. L. Miller, Jr., G. E. Stone, and D. M. Prescott
19. PREPARATION OF MAMMALIAN METAPHASE CHROMOSOMES FOR  
AUTORADIOGRAPHY  
D. M. Prescott and M. A. Bender
20. METHODS FOR MEASURING THE LENGTH OF THE MITOTIC CYCLE AND  
THE TIMING OF DNA SYNTHESIS FOR MAMMALIAN CELLS IN  
CULTURE  
Jesse E. Siken
21. MICRURGY OF TISSUE CULTURE CELLS  
Lester Goldstein and Julie Micou Eastwood
22. MICROEXTRACTION AND MICROELECTROPHORESIS FOR DETERMINATION  
AND ANALYSIS OF NUCLEIC ACIDS IN ISOLATED CELLULAR UNITS  
J.-E. Edström

AUTHOR INDEX—SUBJECT INDEX

## *Volume II*

1. NUCLEAR TRANSPLANTATION IN AMPHIBIA  
Thomas J. King
2. TECHNIQUES FOR THE STUDY OF LAMPBRUSH CHROMOSOMES  
Joseph G. Gall
3. MICRURGY ON CELLS WITH POLYTENE CHROMOSOMES  
H. Kroeger
4. A NOVEL METHOD FOR CUTTING GIANT CELLS TO STUDY VIRAL SYN-  
THESIS IN ANUCLEATE CYTOPLASM  
Philip I. Marcus and Morton E. Freiman

5. A METHOD FOR THE ISOLATION OF MAMMALIAN METAPHASE CHROMOSOMES  
Joseph J. Maio and Carl L. Schildkraut
  6. ISOLATION OF SINGLE NUCLEI AND MASS PREPARATIONS OF NUCLEI FROM SEVERAL CELL TYPES  
D. M. Prescott, M. V. N. Rao, D. P. Evenson, G. E. Stone, and J. D. Thrasher
  7. EVALUATION OF TURGIDITY, PLASMOLYSIS, AND DEPLASMOLYSIS OF PLANT CELLS  
E. J. Stadelmann
  8. CULTURE MEDIA FOR *Euglena gracilis*  
S. H. Hutner, A. C. Zahalsky, S. Aaronson, Herman Baker, and Oscar Frank
  9. GENERAL AREA OF AUTORADIOGRAPHY AT THE ELECTRON MICROSCOPE LEVEL  
Miriam M. Salpeter
  10. HIGH RESOLUTION AUTORADIOGRAPHY  
A. R. Stevens
  11. METHODS FOR HANDLING SMALL NUMBERS OF CELLS FOR ELECTRON MICROSCOPY  
Charles J. Flickinger
  12. ANALYSIS OF RENEWING EPITHELIAL CELL POPULATIONS  
J. D. Thrasher
  13. PATTERNS OF CELL DIVISION: THE DEMONSTRATION OF DISCRETE CELL POPULATIONS  
Seymour Gelfant
  14. BIOCHEMICAL AND GENETIC METHODS IN THE STUDY OF CELLULAR SLIME MOLD DEVELOPMENT  
Maurice Sussman
- AUTHOR INDEX—SUBJECT INDEX

### *Volume III*

1. MEASUREMENT OF CELL VOLUMES BY ELECTRIC SENSING ZONE INSTRUMENTS  
R. J. Harvey



2. SYNCHRONIZATION METHODS FOR MAMMALIAN CELL CULTURES  
Elton Stubblefield
3. EXPERIMENTAL TECHNIQUES FOR INVESTIGATION OF THE AMPHIBIAN  
LENS EPITHELIUM  
Howard Rothstein
4. CULTIVATION TISSUES AND LEUKOCYTES FROM AMPHIBIANS  
Takeshi Seto and Donald E. Rounds
5. EXPERIMENTAL PROCEDURES FOR MEASURING CELL POPULATION  
KINETIC PARAMETERS IN PLANT ROOT MERISTEMS  
Jack Van't Hof
6. INDUCTION OF SYNCHRONY IN *Chlamydomonas moewusii* AS A TOOL  
FOR THE STUDY OF CELL DIVISION  
Emil Bernstein
7. STAGING OF THE CELL CYCLE WITH TIME-LAPSE PHOTOGRAPHY  
Jane L. Showacre
8. METHOD FOR REVERSIBLE INHIBITION OF CELL DIVISION IN *Tetra-  
hymena pyriformis* USING VINBLASTINE SULFATE  
Gordon E. Stone
9. PHYSIOLOGICAL STUDIES OF CELLS OF ROOT MERISTEMS  
D. Davidson
10. CELL CYCLE ANALYSIS  
D. S. Nachtwey and I. L. Cameron
11. A METHOD FOR THE STUDY OF CELL PROLIFERATION AND RENEWAL  
IN THE TISSUES OF MAMMALS  
Ivan L. Cameron
12. ISOLATION AND FRACTIONATION OF METAPHASE CHROMOSOMES  
Norman P. Salzman and John Mendelsohn
13. AUTORADIOGRAPHY WITH THE ELECTRON MICROSCOPE: PROPERTIES OF  
PHOTOGRAPHIC EMULSIONS  
D. F. Hülser and M. F. Rajewsky
14. CYTOLOGICAL AND CYTOCHEMICAL METHODOLOGY OF HISTONES  
James L. Pipkin, Jr.
15. MITOTIC CELLS AS A SOURCE OF SYNCHRONIZED CULTURES  
D. F. Petersen, E. C. Anderson, and R. A. Tobey

*Volume IV*

1. ISOLATION OF THE PACHYTENE STAGE NUCLEI FROM THE SYRIAN  
HAMSTER TESTIS  
Tadashi Utagaki
2. CULTURE METHODS FOR ANURAN CELLS  
Jerome J. Freed and Liselotte Mezger-Freed
3. AXENIC CULTURE OF *Acetabularia* IN A SYNTHETIC MEDIUM  
David C. Shephard
4. PROCEDURES FOR THE ISOLATION OF THE MITOTIC APPARATUS FROM  
CULTURED MAMMALIAN CELLS  
Jesse E. Sicken
5. PREPARATION OF MITOCHONDRIA FROM PROTOZOA AND ALGAE  
D. E. Buetow
6. METHODS USED IN THE AXENIC CULTIVATION OF *Paramecium aurelia*  
W. J. van Wagtenonk and A. T. Soldo
7. PHYSIOLOGICAL AND CYTOLOGICAL METHODS FOR  
*Schizosaccharomyces pombe*  
J. M. Mitchison  
APPENDIX (CHAPTER 7): STAINING THE *S. pombe* NUCLEUS  
C. F. Robinow
8. GENETICAL METHODS FOR *Schizosaccharomyces pombe*  
U. Leupold
9. MICROMANIPULATION OF AMEBA NUCLEI  
K. W. Jeon
10. ISOLATION OF NUCLEI AND NUCLEOLI  
Masami Muramatsu
11. THE EFFICIENCY OF TRITIUM COUNTING WITH SEVEN  
RADIOAUTOGRAPHIC EMULSIONS  
Arie Ron and David M. Prescott
12. METHODS IN PARAMECIUM RESEARCH  
T. M. Sonneborn
13. AMEBO-FLAGELLATES AS RESEARCH PARTNERS: THE LABORATORY  
BIOLOGY OF *Naegleria* AND *Tetramitus*  
Chandler Fulton

14. A STANDARDIZED METHOD OF PERIPHERAL BLOOD CULTURE FOR  
CYTOGENETICAL STUDIES AND ITS MODIFICATION BY COLD  
TEMPERATURE TREATMENT  
Marsha Heuser and Lawrence Razavi
  15. CULTURE OF MEIOTIC CELLS FOR BIOCHEMICAL STUDIES  
Herbert Stern and Yasuo Hotta
- AUTHOR INDEX—SUBJECT INDEX

## *Volume V*

1. PROCEDURES FOR MAMMALIAN CHROMOSOME PREPARATIONS  
T. C. Hsu
2. CLONING OF MAMMALIAN CELLS  
Richard G. Ham
3. CELL FUSION AND ITS APPLICATION TO STUDIES ON THE  
REGULATION OF THE CELL CYCLE  
Potu N. Rao and Robert T. Johnson
4. MARSUPIAL CELLS *in Vivo* AND *in Vitro*  
Jack D. Thrasher
5. NUCLEAR ENVELOPE ISOLATION  
I. B. Zbarsky
6. MACRO- AND MICRO-OXYGEN ELECTRODE TECHNIQUES FOR  
CELL MEASUREMENT  
Milton A. Lessler
7. METHODS WITH *Tetrahymena*  
L. P. Everhart, Jr.
8. COMPARISON OF A NEW METHOD WITH USUAL METHODS FOR  
PREPARING MONOLAYERS IN ELECTRON MICROSCOPY  
AUTORADIOGRAPHY  
N. M. Maraldi, G. Biagini, P. Simoni, and R. Laschi
9. CONTINUOUS AUTOMATIC CULTIVATION OF HOMOCONTINUOUS  
AND SYNCHRONIZED MICROALGAE  
Horst Senger, Jürgen Pfau, and Klaus Werthmüller
10. VITAL STAINING OF PLANT CELLS  
Eduard J. Stadelmann and Helmut Kinzel
11. SYNCHRONY IN BLUE-GREEN ALGAE  
Harald Lorenzen and G. S. Venkataraman

AUTHOR INDEX—SUBJECT INDEX

# CONTENTS

LIST OF CONTRIBUTORS

PREFACE

CONTENTS OF PREVIOUS VOLUMES

## 1. *Cultivation of Cells in Protein- and Lipid-Free Synthetic Media*

*Hajim Katsuta and Toshiko Takaoka*

I. Significance of Culturing Cells in Synthetic Media	1
II. Composition of Chemically Defined Synthetic Media	2
III. Cell Lines Serially Grown in Synthetic Media	5
IV. Initiation of Cell Growth in Synthetic Media	13
V. Biomorphological Changes Induced in Cells by Transfer to Synthetic Media	18
VI. Tumorigenicity of Cells Grown in Synthetic Media	32
VII. Sensitivity of Cells Grown in Synthetic Media to Light Irradiation, Chemical Carcinogen Treatment, and Deep Freezing	35
VIII. Factors Presumably Responsible for the Failure of Cultivation of Cells in Synthetic Media	38
References	40

## 2. *Preparation of Synchronous Cell Cultures from Early Interphase Cells Obtained by Sucrose Gradient Centrifugation*

*Richard Schindler and Jean Claude Schaer*

I. Introduction	43
II. Requirements to Be Satisfied by the Method	45
III. Cell Line and Culture Techniques	46
IV. Preparation of Partially Synchronous Cell Populations	48
V. Methods Used for Characterization of Synchronous Cultures	50
VI. Characteristics of Synchronous Cultures	51
VII. Experimental Factors Affecting the Degree of Synchrony	60
VIII. Discussion	62
References	65

## 3. *Production and Characterization of Mammalian Cells Reversibly Arrested in $G_1$ by Growth in Isoleucine-Deficient Medium*

*Robert A. Tobey*

I. Introduction	68
II. Culture Conditions Affecting Induction of $G_1$ Arrest in Isoleucine-Deficient Medium	72

III. Characterization of Cells in Isoleucine-Deficient Medium	82
IV. Utilization of Cells Reversibly Arrested in G <sub>1</sub>	97
V. Summary and Conclusions	107
References	109
4. <i>A Method for Measuring Cell Cycle Phases in Suspension Cultures</i>	
<i>P. Volpe and T. Eremenko</i>	
I. Introduction	113
II. Methods	116
III. Results	118
IV. Concluding Remarks	124
References	125
5. <i>A Replica Plating Method of Cultured Mammalian Cells</i>	
<i>Fumio Suzuki and Masakatsu Horikawa</i>	
I. Introduction	127
II. Procedure for Replica Plating of Cultured Mouse 3T6 Cells and HeLa S3 Cells	128
III. Detection and Isolation of Nutritionally Deficient Mutants from the Original Chinese Hamster <i>Hai</i> Cells by a Replica Plating Method	135
IV. Concluding Remarks	140
References	141
6. <i>Cell Culture Contaminants</i>	
<i>Peter P. Ludovici and Nelda B. Holmgren</i>	
I. Introduction	144
II. Mycoplasma Contamination of Cultured Cells	144
III. Virus Contamination of Cultured Cells	164
IV. Bacteria and Fungi Contamination of Cultured Cells	179
V. Parasite Contamination of Cultured Cells	188
VI. Cell Contamination by Cultured Cells	193
VII. Summary	201
References	202
7. <i>Isolation of Mutants of Cultured Mammalian Cells</i>	
<i>Larry H. Thompson and Raymond M. Baker</i>	
I. Introduction	210
II. Culture Requirements	217
III. Isolation of Drug-Resistant Mutants	227
IV. Isolation of Auxotrophic and Conditional Lethal Mutants by Indirect Selection	246
V. Mutant Frequency and the Effects of Mutagenic Agents	263
VI. The Nature of Somatic Cell Mutants Isolated in Culture	274
References	277

8. <i>Isolation of Metaphase Chromosomes, Mitotic Apparatus, and Nuclei</i> Wayne Wray	
I. Introduction	283
II. General Methods	286
III. Isolation Methods and Properties	288
IV. Discussion	300
References	305
9. <i>Isolation of Metaphase Chromosomes with High Molecular Weight DNA at pH 10.5</i> Wayne Wray	
I. Introduction	307
II. Cell Labeling	308
III. Alkaline Sucrose Gradient Techniques	309
IV. Molecular Weight Analysis of Reported Chromosome Isolation Procedures	309
V. Chromosome Isolation at pH 10.5	309
VI. Discussion	313
References	315
10. <i>Basic Principles of a Method of Nucleoli Isolation</i> J. Zalta and J-P. Zalta	
I. Introduction	317
II. Determination of a Concentration Threshold of Mg Ions ( $Mg^{++}$ ) to Obtain Differential Behavior of Nucleoli and Extranucleolar Chromatin	318
III. Dispersion of Extranucleolar Chromatin and Isolation of Nucleoli	322
IV. Conclusion	324
References	324
11. <i>A Technique for Studying Chemotaxis of Leukocytes in Well-Defined Chemotactic Fields</i> Gary J. Grimes and Frank S. Barnes	
I. Introduction	325
II. Methods and Materials	326
III. Measurements of the Sensitivity of Polymorphonuclear Leukocytes to Cyclic Adenosine-3',5'-monophosphate Concentrations and Gradients	336
IV. Discussion	340
V. Appendix: Techniques for Evaluating the Diffusion Equations	342
References	343
12. <i>New Staining Methods for Chromosomes</i> H. A. Lubs, W. H. McKenzie, S. R. Patil, and S. Merrick	
I. Introduction	346
II. Preparative Techniques: A Reevaluation	354

III. Principal Methods: Original and Recommended Procedures	357
IV. Special Procedures and Troubleshooting	367
V. Mechanisms of Banding	374
VI. Significance and Applications of the New Techniques	376
References	379
AUTHOR INDEX	381
SUBJECT INDEX	395

# Chapter 1

## *Cultivation of Cells in Protein- and Lipid-Free Synthetic Media*

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I. Significance of Culturing Cells in Synthetic Media . . . . .	1
II. Composition of Chemically Defined Synthetic Media . . . . .	2
III. Cell Lines Serially Grown in Synthetic Media . . . . .	5
IV. Initiation of Cell Growth in Synthetic Media . . . . .	13
A. Prompt Initiation of Growth without Special Treatment . . . . .	13
B. Gradual Adaptation to Synthetic Media with No Supplement . . . . .	17
C. Very Gradual Adaptation with Decrease in Concentration of Macromolecular Substances . . . . .	17
V. Biomorphological Changes Induced in Cells by Transfer to Synthetic Media . . . . .	18
VI. Tumorigenicity of Cells Grown in Synthetic Media . . . . .	32
VII. Sensitivity of Cells Grown in Synthetic Media to Light Irradiation, Chemical Carcinogen Treatment, and Deep Freezing . . . . .	35
VIII. Factors Presumably Responsible for the Failure of Cultivation of Cells in Synthetic Media . . . . .	38
References . . . . .	40

### I. Significance of Culturing Cells in Synthetic Media

Protein-free, chemically defined synthetic media are of great use in analyzing the chemical nature of cultured cells and of the metabolites excreted from them as well as in examining the effect of certain substances on cells, especially in cases where the substances may first interact with serum proteins in the medium. A number of studies have been carried out along these lines, as will be discussed later. However, a very limited number of kinds of cell lines have been grown indefinitely in protein-free media.



## II. Composition of Chemically Defined Synthetic Media

Many mixtures of synthetic media have been reported. Table I lists the names of mixtures and authors of synthetic media, so far as known to us, which have been designed for the cultivation of mammalian cells. Different kinds of cells naturally have different nutritional requirements. In addition, our knowledge of cell metabolism is very limited. These are among the reasons why descriptions of so many mixtures have been published and not every kind of cell has as yet been serially grown in such media, especially in the primary culture.

Some of these mixtures were designed theoretically, all others empirically. Our work on synthetic media stemmed from our question of whether high molecular weight substances in media might be essential for cells as a nutritional source. By the use of primary culture of rat ascites hepatoma AH-130 cells, we estimated the consumption of serum proteins in the medium by the cells following cultivation. However, little decrease was detected in the amount of proteins. The addition of  $^{131}\text{I}$ -labeled serum proteins also revealed little incorporation of the proteins into cells. These findings showed that proteins presumably do not serve cells as nutritional substances. We tried to replace serum proteins in the medium with other high molecular weight substances, especially with so-called plasma expanders, which were developed during the Second World War; alginic acid, dextran, and polyvinylpyrrolidone (PVP). All of them were more or less effective. However, the highest efficiency of substitution was obtained with 0.1% PVP (K-90, average molecular weight 700,000; Badische Anilin- und Soda-Fabrik, West Germany), which replaced approximately 99.5% by volume of serum proteins added to the medium in the optimal concentration (Katsuta *et al.*, 1959a). This result was introduced into the cultivation of L-929 cells in protein-free media. When transferred to medium consisting of 0.05% PVP, 0.4% lactalbumin hydrolyzate (NBCo, U.S.A.), and 0.08% yeast extract (Difco Lab., U.S.A.) with no supplement of serum, the proliferation of L-929 cells was readily initiated and has continued up to the present (Katsuta *et al.*, 1959b). This subline was designated as L·P1. In parallel to this cultivation, other trials were made with other mixtures of protein-free media consisting of (1) PVP and lactalbumin hydrolyzate and (2) lactalbumin hydrolyzate alone. Continuous cell growth in these media was eventually obtained; the sublines grown in (1) and (2) were designated as L·P2 and L·P4, respectively (Katsuta *et al.*, 1961).

Since L-929 cells were demonstrated, as above, to be capable of grow-