Cell Physiology

Molecular Dynamics

CELL PHYSIOLOGY

Molecular Dynamics

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ACADEMIC PRESS New York and London

A Subsidiary of Harcourt Brace Jovanovich, Publishers

Cover illustration: Section of timothy grass leaf. Micrograph by S. E. Frederick; courtesy of E. H. Newcomb, University of Wisconsin. (See Fig. 1.7, p. 16.)

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data

Tedeschi, Henry.
Cell physiology: molecular dynamics.

Includes bibliographies.
1. Cell physiology. 2. Molecular biology.
I. Title. [DNLM: 1. Cells–Physiology. QH631 T256c 1974]
QH631.T4 574.8'76 73-9448
ISBN 0–12–685150–6

PRINTED IN THE UNITED STATES OF AMERICA

Preface

"Cell Physiology: Molecular Dynamics" is intended as a text for juniors and seniors who have a basic knowledge of the biochemical and genetic approaches to biology. The book is comprised of three sections. In the first one the salient points of the functional and structural organization of the cell are briefly summarized. In the second and third sections cell physiology from two distinct points of view is discussed. In the second part the cell is regarded as a unit of inheritance, and so this portion of the book is involved with the bases of genetic transmission, the transcription and translation of the genetic message, and the regulation of these processes. In the chapters comprising the third section, the cell is considered as a biochemical transducer. The capture of energy in oxidative phosphorylation and photosynthesis and its utilization in performing biological work such as muscle contraction or transport against an electrochemical gradient are discussed.

An attempt is made to use key experiments to describe basic principles. For example, the principle of semiconservation in DNA duplication is discussed directly by selecting the now classic experiment of Meselson and Stahl. The presentation strives to combine the virtues of the conventional coverage of a textbook and the use of selected excerpts from original articles. The references are sufficient to permit exploration of the literature and are supplemented by Suggested Reading for further study.

It is hoped that the presentation will foster the ability to form views independent of those of either teacher or textbook. The book is designed to encourage students to read and evaluate original articles. Hopefully, this will make them aware of the major issues of each area of cell physiology, enabling them to follow future progress in this rapidly developing field.

I would like to thank my wife, Terry, and my children, Alexander, Devorah and David, for their encouragement and help. I owe thanks to Neena B. Schwartz who convinced me of the need for a modern cell physiology textbook, and to Walter Damus, Daniel L. Harris, and Raul Parodi, who many years ago introduced me to the pleasures of the mind. Several of my friends and colleagues were kind enough to read part of the book and offer various suggestions, corrections, and criticisms at one or more of the various stages of its development: Richard W. Barker, Katherine Bowler, John S. Condeelis, Joyce Johnson Diwan, Charles Edwards, David L. Edwards, Millie Ernau, Earl Ettienne, Jerry Feldman, Shelagh Ferguson-Miller, Robert P. Hard, Colin S. Izzard, Sally L. Izzard, Eiji Kamitsubo, Linda R. Lochner, Robert I. Macey, Joseph P. Mascarenhas, Florian A. Muckenthaler, Robert Rikmenspoel, Earl Rollins, and Joseph T. Tupper. I am grateful to the students of the various cell biology classes at SUNYA who taught me a good deal. Jane Sandwick helped with the various compilations and needed correspondence.

Henry Tedeschi

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Part II: THE CELL AS A GENETIC UNIT

PART I

AN INTRODUCTION TO THE CELL

CHAPTER 1

The Cell: A System of Compartments

We think of cells as basic and largely autonomous functional units. In multicellular organisms they can form tissues of considerable complexity where they are responsible for many of the functional specializations of the organism. This view is not entirely correct since there are a number of exceptions. Single-celled organisms (the protists) can exhibit considerable complexity in the specialization of their component parts, and many relatively large organisms may possess a single large, polynucleated cytoplasmic mass as in the case of acellular slime molds. Comparably in more complex organisms, tissue masses may behave as if they were composed of interconnected units. Insect epithelial cells (20) conduct electrical currents as if the component cells were interconnected. Other organisms may actually be formed by large cells, each one a single compartment

of large size; the multinucleated internodal cells of *Nitella,* for example, can be as long as 2 inches.

Nevertheless, in most cases, cells can be considered to be the basic semi-independent units or compartments making up a tissue of greater complexity. The autonomy of the cell depends to a large extent on the presence of semipermeable membrane enclosing the cell. The integrity of its internal machinery depends on the control of its internal medium and on the retention of needed components.

Similarly the interior of the cell is made up of compartments of characteristic chemical composition and functional behavior. These compartments necessarily depend on membranes which separate them from the interior of the cell. In the present discussion we will focus on the general ground plan of cells in terms of these internal compartments.

I. THE GENERAL ORGANIZATION OF THE CELL

Our knowledge of the organization of the cell depends to a large extent on the technical approaches that have been developed for its study. Physical methods, largely microscopy, have allowed us to form some idea of the general blueprint of the organization of the cell. These observations, together with the studies of the molecular makeup of the cell, are bringing us close to understanding many of the processes which take place inside the cell. The sections that follow will discuss the general organization of the cell.

Cells can be viewed using several microscopic methods utilizing visible light, ultraviolet light, or X-rays. A comparison of the principles used in conventional light microscopy (Part a) and in interference or phase-contrast microscopy (Part b) is shown in Diagram 1.1. In visible light the various cell components do not have much contrast when viewed through an ordinary light microscope; therefore cell components often have to be stained unless they contain some natural chemical substance which absorbs light. The specimen is generally fixed with chemicals, or heat, to immobilize the component macromolecules (by cross-linking or denaturing). Often after dehydration, the fixed material is embedded in paraffin or plastic and then sectioned before staining. Conventional cytological sections are routinely made which range in thickness between 5 and 10 μm for paraffin media and 1–2 μm for plastic media. The light microscope can be modified to function as a microspectrophotometer. With this technique, the amount of light absorbed by a dye can be

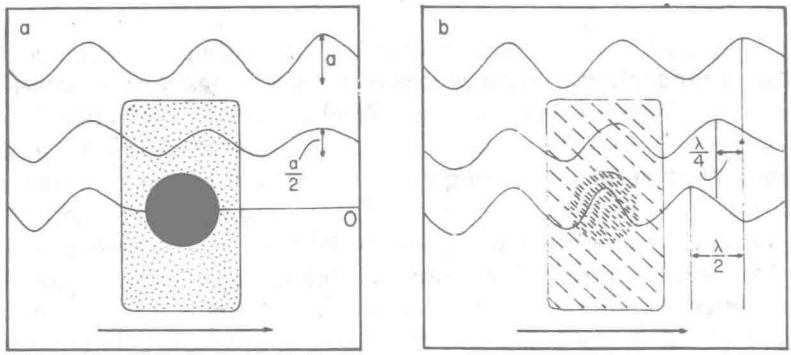

Diagram 1.1. From K. F. A. Ross, "Phase Contrast and Interference Microscopy for Cell Biologist." St. Martin's Press, New York, 1967.

measured quantitatively by a photocell. In this way the chemical composition of the cell components can be estimated with a precision that compares favorably with chemical analytical procedures.

Microscopic techniques are also available to generate a contrast image without recourse to staining the specimen. These methods permit not only studies of living cells in their native state but also allow the dynamics of cells to be followed by means of cinematographic recording. The two kind of techniques are contrasted in Diagram 1.1. Part a represents the passage of light waves through a stained cell in conventional light microscopy. Absorption reduces the amplitude of the light waves. Part b represents the passage through an unstained object. The light passing through the denser nucleus (an object with a higher refractive index) is retarded. The contrast techniques take advantage of light interference optics. When two coherent beams of light are recombined they will interfere with each other, either constructively or destructively, depending on their phase relationship, to produce a contrast image. This principle is used in *phase-contrast, interference,* and *differential interference* (Nomarski) microscopy, but in different ways. As shown in the diagram, the beam passing through the object is retarded; the thicker or the denser the specimen, the greater is the retardation of the beam. In phase-contrast microscopy, the beam diffracted by the object is recombined with a reference beam which has passed through the medium or the background material. The reference beam has been advanced or retarded in phase by the instrument by a quarter wavelength to produce maximal interference. The difference in phase between the two produces the contrast. The contrast will be positive (i.e., the object will be darker than the background) or

negative (i.e., the object will be brighter) depending on whether the optical arrangement produces constructive or destructive interference. In interference microscopy, the phase of the reference beam can be varied in relation to the specimen beam, permitting the measurement of the actual degree of retardation of the beam which has passed through the specimen. The method can be used as a quantitative tool to estimate the dry weight of cells or parts of cells. The Nomarski differential interference microscope is based on the interference between two closely separated points in the object. The beam passing through the specimen is split by a birefringent plate (a modified Wollaston prism). The resulting image is a gradient of the phase difference between adjacent points of the object. Mathematically, the contrast is the derivative of the path differences with respect to distance. The image has a directional contrast resembling a shadow-cast relief map of cellular details. The Nomarski microscope has a higher contrast for structural detail, which is closer to the limit of resolution than any other light microscope. In addition, objects above or below the plane of focus do not interfere with the image. As a result, the image is in effect the equivalent of a section in conventional microscopy. However, in this case, it is an optical section. The system is advantageous because a living cell can be observed without the necessity of preparatory procedures that may alter its organization (see Ref. 2). Nomarski optics are ideally suited to observe objects with well-defined boundaries. A comparison between Nomarski and phase-contrast microscopy is shown in Fig. 1.1. In this figure, the top photomicrograph corresponds to a view of endosperm cells of *Hemanthus katherinae* using Nomarski differential interference microscopy. The bottom view shows the same cell viewed with positive phase contrast. The details are sharp with the Nomarski optics, and each component of the cell is well defined. In contrast, the objects appear more diffuse with the phase microscope. In addition, with the phase microscope, any object which is slightly out of focus is surrounded by halos.

In the remaining part of this section we will trace the organization of fibroblast cells using first phase microscopy with living cells and later various electron microscope procedures. A photograph of a rat embryo cell (a fibroblast) taken with a phase-contrast microscope is shown in Fig. 1.2 (6). In this figure, the nucleus appears as a dense body (n). Long filamentous structures, which at times are spherical, the mitochondria (m) can be seen in the cytoplasm. In most of the cytoplasm, it is possible to see a fine reticular network, the *endoplasmic reticulum* (er). A system of membranes, the *Golgi apparatus*