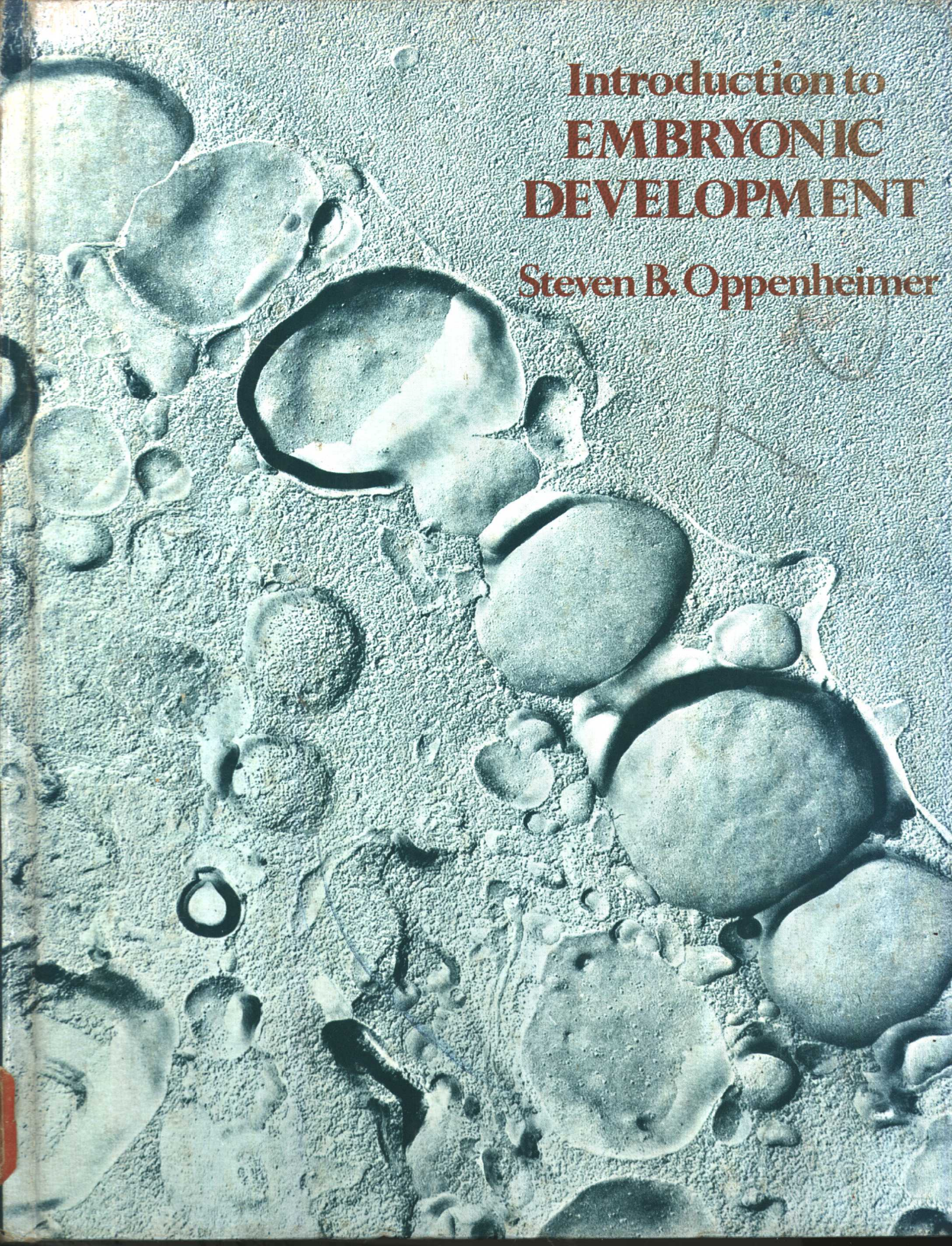

Introduction to EMBRYONIC DEVELOPMENT

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Allyn and Bacon, Inc. Boston London Sydney Toronto

INTRODUCTION TO EMBRYONIC DEVELOPMENT

Library of Congress Cataloging in Publication Data

Oppenheimer, Steven B 1944-
Introduction to embryonic development.

Bibliography: p.
Includes index.
1. Embryology. I. Title. [DNLM: 1. Embryology.
QS604.3 062i]
QL955.066 596'.03'3 79-26339
ISBN 0-205-06899-5

Printed in the United States of America.

Production editor: Mary Beth Finch
Cover designer: Linda Knowles
Preparation buyer: Linda Card
Series editor. Joseph E. Burns

Cover photo:
Freeze fracture of the periphery of an unfertilized sea urchin egg showing cortical granules. Transmission electron micrograph, courtesy of Edward Pollock.

PREFACE

WHEN I UNDERTOOK to write this book, I did so with the hope that sharing my own enthusiasm for the subject matter would excite the curiosity and interest of students approaching the study of embryology and developmental biology. To this end, wherever possible I have introduced topics by asking questions: What turns on the complex series of metabolic reactions that occur in eggs immediately after fertilization? What is the nature of the molecules that control the process of sperm-egg recognition? What are the mechanisms of morphogenesis that shape the embryo? What factors turn on specific genes during differentiation? What is the fascinating relationship between cancer cells and embryonic cells? It is clear that all of the answers to these sorts of questions are not yet at hand. The student, I hope, will be left with more than knowledge of some answers, and will gain an understanding of how the experimental method leads to the answers.

In this text, I have attempted to provide the reader with a logical approach to the subject. The book begins at the beginning—with gametogenesis—and moves through fertilization, cleavage, and each subsequent step in embryonic development. Basic embryology is covered in the first half of the text. With this fundamental background behind the student, the second half of the book examines molecular mechanisms involved in controlling morphogenesis and differentiation. The text ends with a discussion of the intriguing relationship between embryonic cells and tumor cells. The role of the cell surface in controlling developmental events is one major theme stressed throughout the text. The large number of micrographs and drawings should help to convey a concrete sense of work in various aspects of the field. The key terms that appear in the margins, and the glossary at the end of the book, should assist the student in understanding and reviewing the subject matter. The readings suggested at the ends of the chapters have, for the most part, been chosen to help the undergraduate gain a better understanding of the topics discussed.

Acknowledgements. When I began to write this embryology-developmental biology text, I did so with the encouragement of many individuals, including my wife, Carolyn, and my colleagues. With the confidence I received from others and the knowledge that I, myself, have had some of the best teachers in the country who have given me insights into teaching the subject, I decided to undertake the venture. At this time, I wish to thank Heinrich Ursprung, Malcolm Steinberg, Michael Edidin, Saul Roseman, Stephen Roth, and Robert DeHaan, who served as the nucleus of individuals at Johns Hopkins who provided me with the foundations needed to write this text, and helped mold my approach to the subject.

Special thanks are given to Gary Folven, Managing Editor, who skillfully steered this book through the critical final stages, and to Joseph Burns, Editor, who also did a great deal in the final phases involved in the production of this book. I also wish to thank Frank Ruggirello, Editor, who provided initial stimulation to me, and Harvey Pantzis who helped get the book started. I thank Mary Beth Finch, Production Editor, for an extremely meticulous job, and the entire staff at Allyn and Bacon for their excellent assistance.

I would like to thank all my colleagues who have kindly provided photos and permission to use illustrations of their work for this text. I am particularly indebted to Peter Armstrong, Patricia Calarco, David Epel, Garth Nicolson, Steven Rosen, and Victor Vacquier for providing collections of superb micrographs; their time spent in the darkroom to enhance my text is truly appreciated. I would also like to express my gratitude to George Morris of Scientific Illustrators, who did an especially excellent job of drawing numerous figures contained in this text.

I would like to make special mention and express my gratitude to the many fine reviewers who have helped shape and guide me in the writing of this book: Patricia G. Calarco, University of California, San Francisco; David Epel, Hopkins Marine Station, Stanford University; John Morris, Oregon State University; Garth Nicolson, University of California, Irvine; Charles W. Porter, San Jose State Unviersity; Ralph S. Quatrano, Oregon State University; J. R. Shaver, Michigan State University; Fred Wilt, University of California, Berkeley. I have taken much of the advice of these reviewers. Any errors, however, are my own. Finally, I wish to thank my wife Carolyn for excellent typing assistance and a superb sense of humor.

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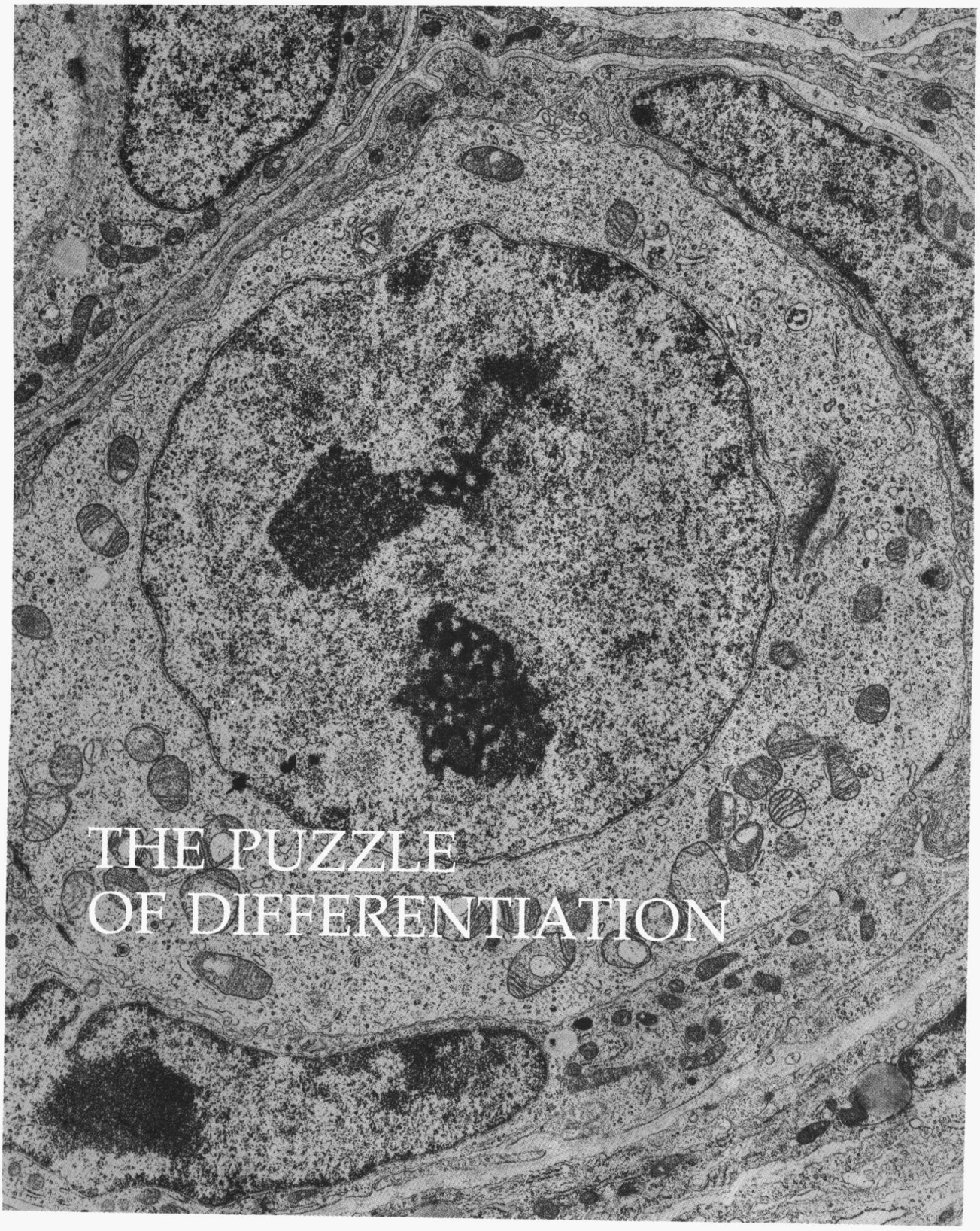

THE PUZZLE OF DIFFERENTIATION

YOU AND I BEGAN LIFE as fertilized eggs (zygotes). Fertilized eggs each contain a set of chromosomes, half from the mother and half from the father. As the fertilized egg begins to divide (cleave) the chromosomes duplicate themselves and all daughter cells usually receive identical sets of chromosomes. The chromosomes contain genes—DNA units that code for the synthesis of messenger RNA. The messenger RNA, in turn, directly codes for the synthesis of specific proteins. We as students of modern biology realize that if all daughter cells usually receive identical sets of chromosomes during early embryonic development, they also receive identical sets of genes. How, then, can cells with the same genes become different? We have muscle, nerve, fat, blood, bone, all different tissues. The "puzzle of differentiation" is one of the most intriguing questions facing embryologists and developmental biologists today.

The question of how cells differentiate, or become different, has, in part, been solved. Much, however, is still to be learned. Throughout this text we will consider the problem of differentiation in different systems and at different levels. First, we will look at the problem superficially to gain some insight into some of the factors that allow cells with identical genes to form all of the tissues necessary for us to function as living organisms.

Cells become different because only certain genes become activated in certain cells. For example, although all of our cells contain the gene that codes for the protein hemoglobin, only red blood cells contain hemoglobin. Thus the hemoglobin genes must have become activated in the red blood cells to allow this cell to produce hemoglobin messenger RNA and, in turn, hemoglobin. In nerve cells or muscle cells, however, although they possess the hemoglobin gene, this gene is inactive and hemoglobin is not synthesized.

What are the factors that activate different genes in different cells? How do these factors work? The first question can, in part, be answered. The answers to the second question, however, are not well understood. The factors that activate specific genes include interaction of the genes with: (1) molecules contained in specific regions of cytoplasm, (2) molecules provided by interaction with neighboring cells, and (3) environ-

mental factors. Let us briefly examine these three factors here. We will return to more in-depth discussions on the nature of differentiation throughout the text.

Interaction of Genes with Molecules in Specific Regions of Cytoplasm

If all daughter cells in developing embryos usually receive the same genes during division of the fertilized egg, don't they also receive the same cytoplasm during the division process? The answer to this question is no, not necessarily. Daughter cells can receive different types of cytoplasm and therefore can receive different molecules that may interact with their genes. One very clear example of how cells in early embryos can receive different cytoplasm is illustrated in Figure I–1. In eggs of organisms such as *Dentalium*, one can distinguish three layers of cytoplasm: a clear layer, a granular layer, and a second clear layer. Before the first division of the fertilized *Dentalium* egg one of the clear cytoplasmic regions is extruded from the zygote (fertilized egg). This extrusion is called a polar lobe. The first division (cleavage) then occurs and the polar lobe containing all of one of the clear regions of cytoplasm is drawn back into only one of the daughter cells (CD cell). Thus, only one of the daughter cells contains this specific cytoplasm. This type of cytoplasmic segregation continues to occur during *Dentalium* development, resulting in the parceling out of specific cytoplasm to specific cells in the embryo. So we can see that although all daughter cells may contain the same

polar lobe

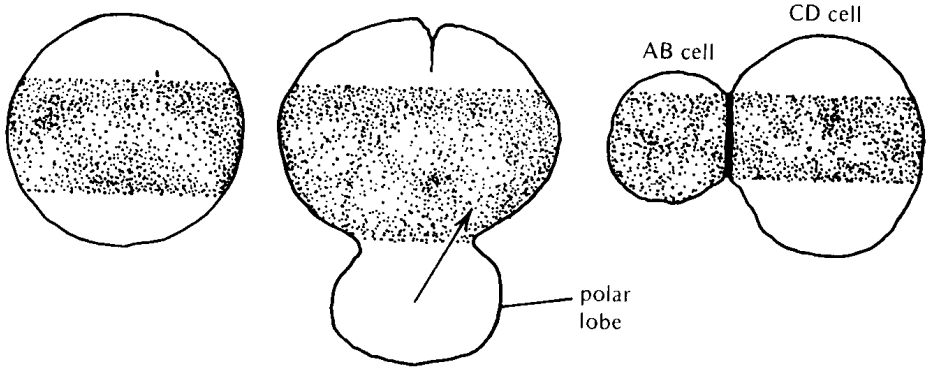

Figure I–1. Cleavage of the mollusc *Dentalium*. After E. B. Wilson, *J. Exp. Zool.* 1:1-72 (1904).

genes, they do not contain the same cytoplasm. The special cytoplasm contained in some of the daughter cells of the embryo appears to play an important role in turning on specific genes in these cells. Only the cells with the special cytoplasm are able to form normal embryos if separated from the other cells. The special cytoplasm of a cell may therefore help that cell become different from other cells by activating specific genes. Evidence to support this statement will be presented in Chapter 8.

We have looked at only one example of cytoplasmic specialization. Later on we will examine other examples, such as the gray crescent of amphibian embryos. Gray crescent cytoplasm is special material localized in only one region of the fertilized egg, needed by specific amphibian embryo cells to differentiate into specific tissue that plays a key role in controlling normal development of the embryo. We will examine these specializations in detail in Chapter 8.

Interaction of Genes with Molecules Provided by Interaction with Neighboring Cells

We saw how special cytoplasm is parceled out to only certain cells in the embryo, causing differences in cells that contain similar genes. Let us now briefly consider an example of a situation in which contact with other cells can cause differentiation to occur in the responding cells. Many examples of this type of cell-cell interaction will be described throughout the text. Here we will consider one of these examples, the amphibian embryo. We just mentioned that the gray crescent in the amphibian embryo appears to contain special cytoplasm. This gray crescent material becomes localized in a specific region of the amphibian embryo that begins to migrate from the surface to the inside of the embryo. This region (the prospective notochord) finally underlies the prospective nervous system of the embryo. Thus the special cytoplasm contained in the original gray crescent is now found in cells that underlie the area that will become the nervous system (Figure I–2). Unless contact is made between the prospective notochord and prospective nervous system, the nervous system does *not* develop. If a sheet of mica is placed between the prospective notochord and prospective nervous system, preventing contact of the two regions, nervous system differen-

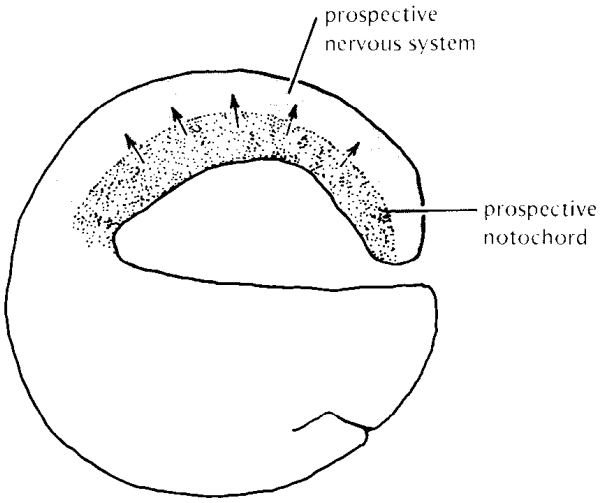

Figure I–2. Contact of two regions in amphibian embryo. Prospective notochord and prospective nervous system (only areas shown) must contact each other for nervous system differentiation to occur.

tiation (formation of the brain, spinal cord, and accessory structures) does not take place. In Chapter 8, we will examine the interaction between these regions in greater detail. We can conclude that in order for the genes that cause nervous system differentiation to become activated in the prospective nervous system cells, interaction with prospective notochord cells must occur. This is an example of differentiation caused by interaction with other cells. The nature of this interaction at a molecular level, however, is not yet well understood.

Environmental factors such as light and temperature can also influence differentiation. Flowering in plants, for example, is controlled by length of light exposure. The development of flowers, in turn, controls the whole reproductive process in plants. Insect development is clearly influenced by light and temperature. As will be seen in Chapter 8, nervous system differentiation can also be influenced by environmental factors such as acidity and salt concentration.

We have introduced embryology by briefly examining cell differentiation caused by rather poorly understood components in special cytoplasm, neighboring cells, and the environment. We will return to this theme in more depth throughout this text to help understand some of the events that occur during the development of different parts of the organism. Toward the end of the text we will return to study differentiation at the molecular level, after the component processes of development have been explored.

In Chapter 1, we will start our study of embryology at the beginning—with the gametes, the sperm and egg, that unite to form the new organism. A study of the fertilization process will help us to understand the complex series of events that must occur before life begins as a divid-

ing embryo. We will continue the study of embryology by examining the factors involved in determining how fertilized eggs cleave. An examination of the events that transform the early embryo, a simple ball of cells, into the complex being that begins to resemble the adult, will follow. Throughout the text, emphasis will be placed upon attempts at understanding the mechanisms involved in controlling the component processes involved in embryonic development. For example, how do cells rearrange themselves in embryos to get their final destinations? Evidence will be presented that suggests that cellular rearrangements in embryos are, in part, caused by adhesive recognition between the cells. Cells stop migrating because they specifically adhere tenaciously to certain other cells. We will examine what is known about the molecular nature of such cell-cell interactions in embryos.

In this text, we will deal with many intriguing questions. How does an egg cell grow to 100,000 times its original volume in preparation for nourishing the new embryo? How does a sperm cell become transformed from a little sphere into a sleek swimming gamete that is able to touch home to fertilize the egg? What turns on the complex series of metabolic reactions that occur in eggs immediately after fertilization? How can a tiny sperm that fuses with only 0.0002 percent of the egg surface trigger the multitude of changes that occur in the new zygote? What is the nature of the molecules involved in the process of sperm-egg recognition? What controls cleavage? How does a conglomeration of cells become transformed into a layered embryo that begins to resemble a "real" organism? What structures are derived from each germ layer and what is the mechanism of their formation? What are the forces that shape the embryo—the mechanisms of morphogenesis? What is the nature of the molecules that appear to "turn on" specific genes during differentiation? How do amphibian limbs regenerate and might this information eventually help us develop means of promoting regeneration of limbs in man? How do our eyes, heart, kidneys, and limbs develop? How does German measles virus cause blindness in human babies? How does one go about studying differentiation? How have modern techniques in molecular, cell, and developmental biology been used to examine the nature of differentiation? How does differentiation at the protein level come about? How are higher orders of structure such as microtubules, microfilaments, flagella, and ribosomes assembled in differentiating cells? What is the nature of the intriguing relationship between cancer cells and embryonic cells? Under what conditions will certain tumor cells become transformed into normal differentiating tissue?

This text will not answer all of these questions completely. We will, however, deal in depth with these questions and many more in what is hoped to be an organized, clear and enthusiastic manner. Some of you

may become excited about this field and eventually participate in uncovering the unsolved problems that still remain in developmental biology.

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