Mechanisms of development

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With 1086 illustrations

THE C. V. MOSBY COMPANY

ST. LOUIS • TORONTO • LONDON 1980

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Printed in the United States of America

The C. V. Mosby Company
11830 Westline Industrial Drive, St. Louis, Missouri 63141

Library of Congress Cataloging in Publication Data

Ham, Richard G 1932-
Mechanisms of development.

Bibliography: p.
Includes index.
1. Developmental biology. I. Veomett, Marilyn J., 1946- joint author. II. Title.
QH491.H33 574.3 79-9236
ISBN 0-8016-2022-8

C/VH/VH 9 8 7 6 5 4 3 2 1 01/C/087

PREFACE

☐ *Mechanisms of Development* was written specifically to serve as the textbook for a one-semester course on developmental mechanisms. Its emphasis is on molecular and cellular mechanisms, and we have included only the bare minimum of descriptive embryology that is needed to understand those mechanisms. There already exist a number of good textbooks of descriptive embryology, and we have tried to avoid unnecessary duplication of the material contained in them. Because of this complementary relationship, *Mechanisms of Development* can also be used in conjunction with a standard embryology text for a comprehensive two-semester course that emphasizes both developmental phenomena and the mechanisms that underlie them.

A living organism is a hierarchy of organization, beginning with the smallest subatomic particles and progressing through atoms, molecules, macromolecules, organelles, cells, tissues, organs, and finally to the intact organism as an integrated functional unit. Our approach in this book is to seek an explanation for each aspect of development at the most basic level of organization that directly affects it. Our attempts to explain developmental phenomena are aimed primarily at the levels of macromolecules, subcellular organelles, cells, and interactions among cells. We also recognize the importance of events at atomic and subatomic levels in determining the properties of the molecules that comprise living organisms, but in most cases we have chosen to view these events as part of the intrinsic nature of the molecules and thus more appropriately covered in texts on atomic physics or chemistry.

Mechanisms of Development is designed for use by upper division undergraduates in the biological sciences. We have assumed that the students using this book have completed a broadly based course in general biology and that they have a reasonable understanding of the fundamentals of chemistry. A number of concepts from molecular biology, biochemistry, and cell biology are essential for a detailed understanding of the mechanisms of development, and students who have completed those courses will find them helpful. However, to make our book comprehensible to students who may not have completed courses in some of these areas, we have also attempted to explain the basic concepts as they are needed, either in the text or in appendixes.

Our definition of development as "progress of an organism through its life cycle" (Chapter 1) is very broad. We are concerned with all phases of the life cycles of multicellular organisms, including gametogenesis, fertilization, embryogenesis, maturation, and senescence. To fit all these topics into a single textbook of manageable size, we have found it necessary to be selective in several ways. First, we have emphasized the biological mechanisms by which development occurs rather than the specific details of development. Second, our presentation is greatly biased toward the development of higher vertebrates, including humans. Other types of organisms are discussed when they are useful to illustrate particular points, but no attempt has been made to describe their development in detail. Third, we have given the greatest attention to those aspects of development for which research into mechanisms has been particularly active. This has resulted in strong emphasis on such areas as the molecular biology of gene expression, the behavior of differentiated cells in culture, and the generation of shape during embryonic development.

Developmental biology is a dynamic, rapidly changing field in which important new discoveries are constantly being made. We have attempted to capture the excitement of new discovery in this book. To do so, we must deal with areas of research in which the final answers are not yet available and, in some cases, in which the investigators are still looking for the right questions to ask. This may be disconcerting to students who are accustomed to textbooks that present their subject material as a compilation of facts and principles. However, we consider it far more important to bring our students to the frontiers of new research than to pretend that we already know everything worth knowing about the mechanisms of development.

Since we are dealing with current research, it is inevitable that some of the material in this book will be obsolete even before the book is off the press. We have tried to identify in the text those areas which we consider likely to change rapidly. In addition, we have included an appendix showing the interested student how to use the research literature to keep abreast with the latest progress in the field.

We consider it desirable for our readers to have some knowledge of overall patterns of development before attempting to understand the detailed mechanisms that are involved. The first section of this book therefore provides an overview of development and a brief description of the major phenomena that are involved. The first chapter presents our working definitions of "mechanism" and "development," together with a summary of the types of life cycles that occur in various organisms and the relationship between those life cycles and development. The second chapter provides a summary of the major events in early embryonic development, and the third chapter introduces the concept of cellular differentiation.

After this brief introduction attention is turned to basic control mechanisms that affect development at the molecular level of organization. Section Two describes molecular control of gene expression in considerable detail. Since many of the pioneering studies in that area have been done with prokaryotic organisms, the first chapter in the section provides a brief review of that work, including both the basic mechanisms of gene expression and the control systems that are responsible for switching specific genes on and off in prokaryotic cells. The next five chapters examine the molecular pathway of information flow in eukaryotic cells, starting with the organization of the genome and attempting to follow the information all the way to final differentiated products. The emphasis of this section is on the mechanisms believed to be responsible for regulating the expression of specific genes in differentiated cells.

Section Three deals with differentiation as a cellular phenomenon. The first two chapters are concerned with environmental influences that control the expression of differentiation in cultured cells and with possible mechanisms for such controls. Attention is then shifted to control mechanisms operating in the intact embryo, including intrinsic cellular determinants, embryonic induction, and hormonal regulation. Throughout this section the emphasis is on the nature of signals that a cell receives from its environment and on the intracellular changes triggered by those signals that ultimately lead to the expression of specific differentiated properties.

Section Four deals with the generation of specific form and shape during development. This section begins with a discussion of how cells change shape and how such changes can influence the shapes of tissues and organs. Attention is then given to mechanisms of cellular recognition, sorting, and aggregation. The last two chapters in the section deal with selective growth control and selective cell death as mechanisms that help to mold the final shape of the organism.

The separation of differentiation and morphogenesis in Sections Three and Four was introduced for simplicity of presentation and does not exist in real life, where the two are intimately intertwined. This is clearly illustrated in Section Five, where selected developmental systems are analyzed in terms of a variety of the mechanisms previously presented. The first chapter in this section deals with gametogenesis and fertilization and serves both to illustrate a high level of cellular adaptation for specialized function and to add material concerning parts of the life cycle that have received relatively little attention in other chapters. The second chapter is concerned with mammalian sex differentiation, a process that offers a fascinating example of hormonal control of development in many diverse tissues and at all levels of organization from molecules to whole-body morphology. The next chapter deals with vertebrate limb development, a prime example of a system in which multiple inductive interactions generate a complex structure from a rudimentary precursor with a minimum of external influence. This is followed by a chapter on insect development, which is of particular interest because of the extreme experimental manipulations to which insect embryos can be subjected and because it is one of the few developmental systems in which genetic techniques have been extensively used. Next is a chapter on the cellular slime mold *Dictyostelium discoideum*, an organism that provides an unusual example of conversion from individual free living cells to a differentiated multicellular organism and that has also been the subject of detailed studies on the molecular control of gene expression. The section ends with an analysis of aging, which many investigators are beginning to view as a part of an overall program for the entire life cycle and therefore inseparable in principle from embryonic development and maturation.

The final section attempts to bring together into a coherent picture all the developmental mechanisms that have been presented. In addition, it seeks to summarize some of the major unanswered questions about development that are now capable of being approached with modern analytical techniques of molecular and cellular biology. We expect that many of these questions will be answered as today's students become tomorrow's investigators. Hopefully this book will inspire some of its readers to take a closer look at what we do not yet know about development.

We thank Karen Brown for preparing the manuscript, Susan Jennings and Lisa Klaumann for doing library research and locating previously published figures, and Paul Ham and Cathy Verhulst for providing original illustrations. We are indebted to the many authors and publishers who have given us permission to reproduce their previously published illustrations. Thanks are also due the many people who have read and criticized portions of the manuscript, including Dr. Mary Bonneville, Dr. Dona Chikaraishi, Dr. Kathleen Danna, Dr. John David, Ms. Kaye Edwards, Dr. Larry Gold, Ms. Kathy Jones, Dr. Edwin McConkey, Dr. J. Richard McIntosh, Dr. Donna Peehl,

Dr. Keith Porter, Dr. Donald Riddle, Dr. Urs Rutishauser, Mr. Gary Shipley, Dr. James R. Smith, Dr. Larry Soll, Dr. Jonathan Van Blerkom, Dr. Ben Walthall, and Dr. Michael Yarus. We particularly thank Dr. George Veomett for many helpful suggestions and for the preparation of Appendix C. Special thanks are also due the 1976, 1977, and 1978 classes in developmental mechanisms at the University of Colorado for serving as experimental subjects and providing the feedback that helped this book to develop into its current form. Finally, we thank Mary, George, and our respective children for putting up with our preoccupation during the long gestation period of this book.

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CONTENTS

SECTION ONE

Basic concepts

□ The detailed analysis of the mechanisms of development presented in subsequent sections of this book begins at the molecular level of organization and moves progressively to more and more complex developmental phenomena. There is an inherent danger in this approach: the details may completely obscure the overall phenomena that we are trying to explain. This introductory section seeks to minimize that risk by providing a brief overview of developmental biology, first at the level of the intact organism and then in terms of the organism's component parts.

One of the basic tenets of information theory is that the symbols used for communication have no inherent meaning in themselves. Any symbolic code, whether it is the number of lanterns in a church steeple, a series of dots and dashes, or the words on a printed page, fails to communicate until everyone involved has agreed to use a common set of definitions. If we, the authors, use a word such as "development" to mean one thing and our readers understand it to mean something different, communication will be seriously impaired. We have therefore chosen to use a substantial part of Chapter 1 to define as precisely as possible concepts such as "mechanism" and "development" and to be certain that we have established meaningful communication.

In addition to presenting basic concepts and definitions, the first chapter also contains a discussion of the continuity of life from generation to generation and of the role of developmental processes in maintaining that continuity. Chapter 2 focuses on the individual organism and on the generation of specific shapes (morphogenesis) as each new organism is formed from a fertilized egg cell. Chapter 3 shifts to the cellular level and focuses on the diversity of shape and function that the various cells within each organism exhibit (cellular differentiation) and on the biochemical and ultrastructural bases for those differences.

Together these three chapters provide a road map to help the reader remain oriented during the detailed analysis of selective gene expression, cellular differentiation, and morphogenesis in later sections of this book.

CHAPTER 1

Life cycles and development

☐ The words "mechanism" and "development" are both used in many different contexts in the English language, and each has a long list of dictionary definitions. Even within the field of developmental biology, it sometimes seems as though there are nearly as many definitions of development as there are developmental biologists. In this chapter we present the rationale behind our own definitions of "mechanism" and "development," which are, respectively, "the fundamental details of how things work" and "progress of an organism through its life cycle." Together these two definitions state accurately the aim of this book, which is to summarize at molecular and cellular levels of organization what is known about the events responsible for the sequence of changes that living organisms go through during their life cycles. Later in this chapter we also delve into the various types of life cycles that exist and the major developmental changes that occur during those cycles.

MECHANISMS

Definitions from three different dictionaries provide a starting point for our attempts to convey exactly what we mean when we use the word "mechanism" in this book: (1) "the means or way in which something is done" (*World Book Encyclopedia Dictionary*), (2) "the agency or means by which an effect is produced or a purpose is accomplished" (*Random House College Dictionary*); (3) "the fundamental physical or chemical processes involved in or responsible for an action, reaction, or other natural phenomenon" (*Webster's New Collegiate Dictionary*). These definitions suggest that the overall concept of a mechanism includes both the means or machinery involved in a process and the way that the machinery functions to achieve the desired results. In simpler terms, what we are talking about is "how things work."

To understand the mechanisms involved in a particular process, it is generally necessary to examine events occurring at more fundamental levels of organization than that of the process itself. For example, to describe the mechanisms responsible for changes in shape and function that are observed in developing embryos, we find it necessary to speak of events that take place at molecular and cellular levels of organization. Similarly, when a chemist explains the mechanisms responsible for reactions that occur at the molecular level, it is necessary to speak in terms of events occurring at atomic and subatomic levels. Thus the simple definition needs to be amended to read "the fundamental details of how things work."

The term "mechanism" also carries an implication of cause and effect. Each event in a developmental sequence tends to be influenced (and in some cases directly controlled) by those which precede it, and each in turn tends to influence those which follow. For example, the release of specific hormones is often triggered by neural factors or by other hormones, and, in turn, the hormones that are released exert a variety of specific effects on their target tissues.

Unfortunately, this type of mechanistic cause-and-effect relationship is often confused with purpose and motivation. A large part of this confusion results from the incorrect use of the word "why" in everyday patterns of speech and thought. When properly used, "why" refers to purpose, reason, or motivation. It can be used unambiguously to refer to voluntary actions or decisions and to refer to the results of such actions. The classical vaudeville question "Why did the chicken cross the road?" and its answer "To get to the other side" illustrate the first case, and the question "Why does this book contain so many definitions?" and its answer "Because the authors consider them important" illustrate the second.

Problems arise when "why" is used to ask a question about processes or things that lack the capability of decision making or voluntary action. One common response to such a question is to substitute immediate cause for motivation and to answer by describing a mechanism. In other words, a "why" question is answered in terms of "how." An example is the question "Why do men have beards and women not?" and the answer "Because their hormones are different." This answer actually describes the mechanism that is responsible for the difference in facial hair between men and women without dealing with cause or motivation in any fundamental sense.

Another common response to inappropriate "why" questions is to answer with teleological reasoning, which assumes (often incorrectly) that all actions are undertaken specifically to achieve goals. An example of this is the question "Why does messenger RNA (mRNA) bind to ribosomes?" and the answer "In order to be translated." In this case the answer implies that the mRNA molecule knows it should be translated and deliberately binds to ribosomes to accomplish that goal. In reality, there is built into the molecular structure of the mRNA an affinity for the ribosomal translation complex, which leads to the binding and subsequent translation of the mRNA (Chapter 9). That affinity is the product of prolonged evolutionary selection for ribosomes and mRNA molecules that work efficiently together and does not involve specific motivation on the part of the interacting molecules. In general, the teleological, or goal-directed, approach is not appropriate for analyzing the basic mechanisms of development, since the cells and molecules that are involved do not make deliberate choices or pursue specific goals.

We have deliberately avoided use of the word "why" in this book. True "why" questions that seek the fundamental reasons or causes for life and the universe being as they are go beyond the realm of experimental science and thus have no place here. Questions about mechanisms and cause-and-effect relationships are better asked in terms of "how" or "What is the mechanism responsible for?" The only time that we consider it legitimate to use "why" is when we are talking about conscious choice or motivation or about actions resulting from them.

DEVELOPMENT

The term "development" has undergone considerable evolution in the hands of developmental biologists, and, as we indicated in the introduction to this section, general agreement on the best definition is still lacking. Two dictionary definitions of the intransitive verb "to develop" provide a starting point for our own efforts: (1) "to grow into a more mature or advanced state" *(Random House)* and (2) "to grow from an embryonic or rudimentary stage into a more complex or adult stage" *(World Book)*. These definitions fit well with everyday experience. Seeds develop into plants; buds develop into flowers; fertilized eggs develop into embryos. But are these definitions complete enough? Are there also other processes in living organisms that should be included?

In one sense all of life is development. Organisms are dynamic and constantly changing. If any living thing ceases developing or changing, it is dead or, at best, dormant. Perhaps the safest definition of development is "progress of an organism through its life cycle." Before we examine the implications of this definition, which is extremely broad, it will be useful to review some of the basic properties of organisms and their life cycles.

CONTINUITY OF LIFE

We are not concerned in this book with the origin of life or with evolution. Within the time scale that we are considering, life comes only from preexisting life of the same kind. What we are concerned with is the ongoing process of reproduction – the formation of new organisms that, except for minor genetic variations, are identical to their parents.

As human individuals, we tend to think of life as finite. We commonly speak of life "beginning" either at conception or at birth and "ending" at the death of the individual. This viewpoint is not adequate for a full understanding of developmental biology. Life is continuous and is passed from one generation to the next in an unbroken chain that lasts as long as the species survives. Each individual is only a temporary link in that chain. We obtain life from our parents and pass it on to our children. This goes on generation after generation. Life is a continuously repeating cycle which, barring extinction, proceeds indefinitely.

LIFE CYCLES

Nature has evolved an overwhelming diversity of life cycles. In every case, however, the overall process is a closed circle. Sometimes cycles have temporary diversions, such as spore formation during adverse environmental conditions, or even alternate cycles, such as a choice between sexual and asexual modes of reproduction. However, if we start with any organism at any stage of its life history (other than postreproductive senescence, which will be discussed later) and follow the progress of that organism and its progeny through subsequent developmental stages, we ultimately observe new individuals at identically the same stage with which we started.

The simplest life cycle consists of growth of an organism, including duplication of all its parts, followed by division into two new organisms, both of which are basically the same as the parent was prior to growth and duplication. Many unicellular organisms, ranging from bacteria to protozoa to cultured human cells, reproduce by this type of asexual cell growth cycle (Fig. 1-1), either exclusively or as one of their available options. In eukaryotic cells such a cycle is referred to as a mitotic cycle, and the division process itself is referred to as mitosis. (The mitotic cycle and methods of analyzing it are described in detail in Appendix C.) The terms "mitosis" and "mitotic cycle" are generally not used for prokaryotic cells, since the chromosomal events characteristic of mitosis (condensation of chromosomes, formation of a mitotic spindle, alignment of chromosomes in a metaphase plate, separation of chromosomes, etc.) do not occur in prokaryotic cells.

Unicellular organisms that reproduce by asexual cell growth cycles provide interesting model systems for the cellular multiplication that occurs during growth of more complex organisms. Also, changes in gene expression that occur during the adaptation of such "simple" organisms to altered environmental conditions are similar in many ways to the process of cellular differentiation that occurs in higher

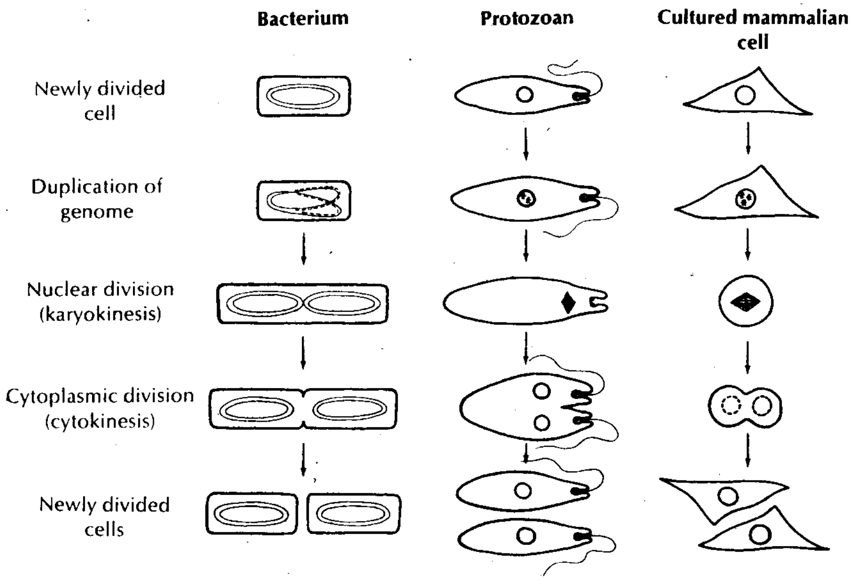

Fig. 1-1. Asexual growth cycles in unicellular organisms. Three very different types of organisms are compared—a procaryotic bacillus, a protozoan, and a cultured mammalian cell (whose behavior within its specialized culture milieu is that of a free-living microorganism). These schematic representations show duplication of the genome, division of the nucleus (or nucleoid in bacteria, which do not have a true nucleus), and, finally, division of the cytoplasm to yield two daughter cells, each of which is essentially the same as the starting cell. These representations are highly schematic and do not illustrate all the complexities that are actually involved. For example, the length of the DNA in the bacterial cell is more than 1000 times the cellular diameter, and a diploid human cell contains 1.74 m of DNA in a nucleus that is only a few microns (10^{-6} m) in diameter.

organisms. The controls over gene expression that operate in bacterial cells provide the starting point for the analysis of gene regulatory mechanisms presented in Section Two of this book and are reviewed in some detail in Chapter 4. Thus, although the primary emphasis in this book is on multicellular organisms, information regarding unicellular forms that reproduce primarily by asexual division is also important to our overall understanding of developmental mechanisms.

In most of the life cycles of more complex organisms the parental organism is partitioned unequally to generate immature progeny that undergo some kind of maturation (other than doubling of size and duplication of parts) to become equivalent to the parent(s). In some cases, such as the colonial alga, *Volvox*, the parent is destroyed during the reproductive process, but more commonly the parent retains its identity and can continue to produce progeny.

Most organisms in which a surviving parent can be distinguished from the progeny exhibit the phenomenon of aging. After a period of reproductive activity that can range from less than a day for some insects to thousands of years for some trees, the parental organisms lose their vigor and ultimately die without any obvious external cause. The postreproductive senescent period is not part of the closed cycle of growth and reproduction that maintains the continuity of life from generation to generation. However, the mechanisms involved in aging are not clearly distinguishable from those involved in development or