# INTERACTING SYSTEMS IN DEVELOPMENT

EBERT

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**JAMES D. EBERT**

DEPARTMENT OF EMBRYOLOGY
CARNEGIE INSTITUTION
OF WASHINGTON

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The twentieth century has seen biology come of age as a conceptual and quantitative science. Major functional phenomena rather than catalogues of animals and plants comprise the core of MODERN BIOLOGY; such heretofore seemingly unrelated fields as cytology, biochemistry, and genetics are now being unified into a common framework at the molecular level.

The purpose of this Series is to introduce the beginning student in college biology—as well as the gifted high school student and all interested readers—both to the concepts unifying the fields of biology and to the diversity of facts that give the entire field its unique texture. Each book in the Series is an introduction to one of the major foundation stones in the mosaic. Taken together, they provide an integration of the general and the comparative, the cellular and the organismic, the animal and the plant, the structural and the functional—in sum, a solid overview of the dynamic science that is MODERN BIOLOGY.

# PREFACE

*The conceptual framework of a scientific discipline determines to a large extent the direction of its progress and its ability to integrate the findings of related branches of knowledge into a meaningful body of facts and theories.*
Kaufmann, 1961

The purpose of this book is to present the concepts of developmental biology to beginners. I have tried to convey the spirit and sense of excitement that I find in my own laboratory and classroom. Developmental biology is emerging as a focal field of research; for the first time we are able to approach some of the age-old problems of mankind with confidence that we are moving toward their solution. I have attempted to effect a synthesis of old and new, of tried and untried, ideas; thus the content of the book is not easily classified. It is neither principally descriptive nor experimental; cellular nor molecular. The problems in which we are interested are never exclusively anatomical, biochemical, or physiological. One approach leads naturally to the next.

Embryos of invertebrates and vertebrates share equal billing with bacteria and viruses, fungi, and occasionally, higher plants. The electron microscope, scintillation counter, and ultracentrifuge receive "equal time" with the glass needle and hair loop.

The field of developmental biology is alive; the pot is boiling, but it must be stirred vigorously—and it needs more than a dash of flair and

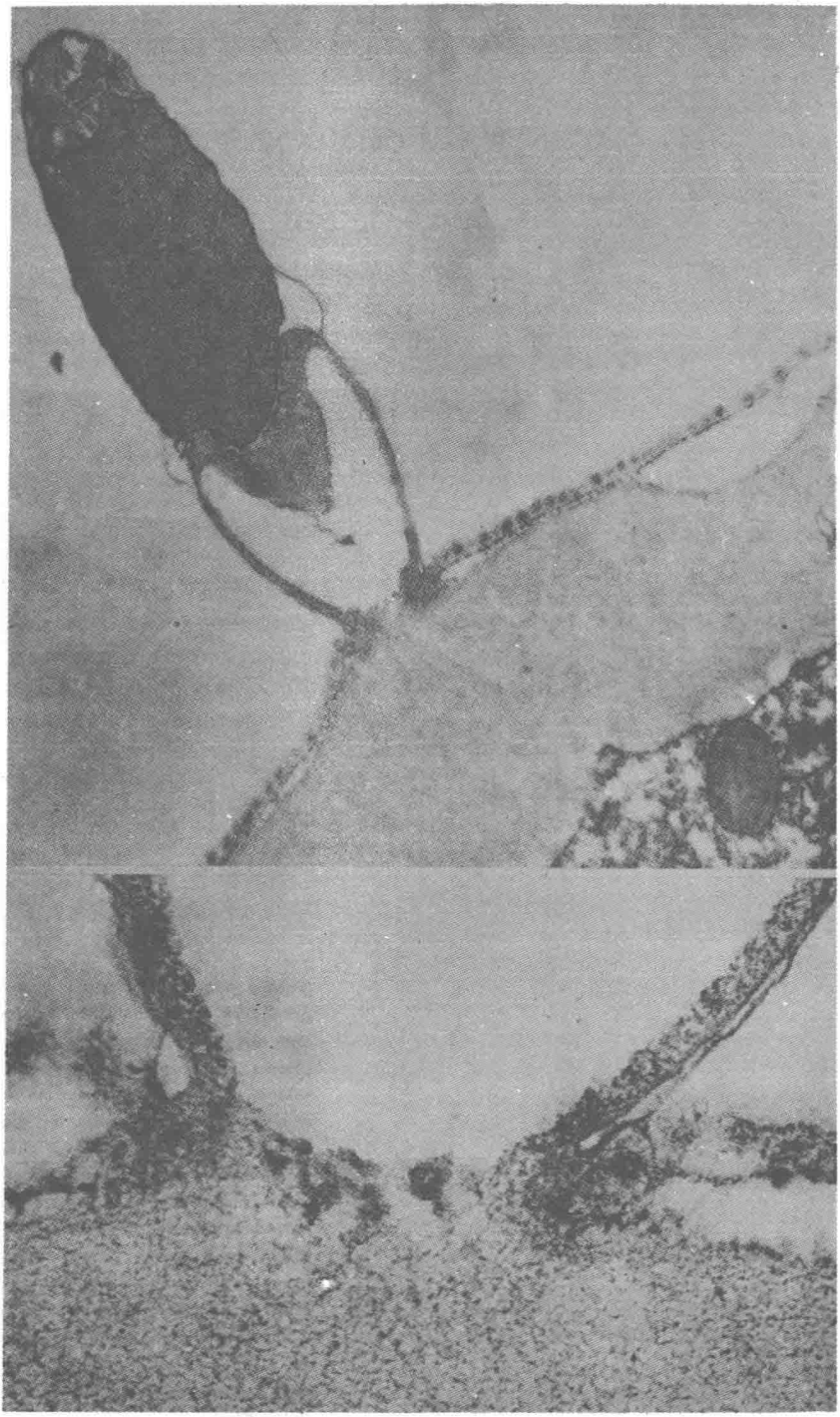
imagination and technical skill. In the next decade these ingredients will be provided by today's beginners. It is essential that from the outset they grasp the concepts of the field and come to grips with the problems confronting us, the origins of these problems, and the framework and intellectual milieu in which they are being approached.

John Coleman, Irving Finger, Ariel Loewy, and Malcolm Steinberg were frank and helpful critics. To them, and to numerous others who helped along the way, I am deeply grateful.

J. D. E.

Baltimore, Maryland
February, 1965

*(Facing page 1).* Electron micrographs of initial contact of sperm and egg membranes. *Top:* Sperm plasma membrane meets egg envelope. Egg plasma membrane is still separated from sperm by egg envelope. x 37,000. *Bottom:* Higher magnification of membrane interaction similar to one shown above. Acrosomal membrane is now inserted into sperm plasma membrane. x 100,000. For details, see Figs. 2–11 and 2–12. (From A. L. Colwin and L. H. Colwin, in *Cellular Membranes in Development,* by courtesy of the authors and Academic Press.)



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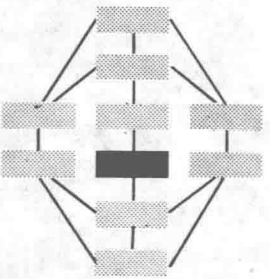

CHAPTER ONE

# INTERACTING SYSTEMS IN DEVELOPMENT

*The body of a worm and the face of a man alike have to be taken as chemical responses. The alchemists dreamed of old that it might be so. Their dream however supposed a magic chemistry. There they were wrong. The chemistry is plain everyday chemistry. But it is complex. Further, the chemical brew, in preparation for it, Time has been stirring unceasingly throughout some millions of years in the service of a final cause. The brew is a selected brew.*

Sherrington, 1951

*Life is a relationship among molecules and not a property of any one molecule.*

Pauling, 1960

Thus, each in his own way, with clarity and grace, Charles Sherrington and Linus Pauling have written the prologue of our introduction to the mechanisms of development.

## THE ORIGIN OF CELL AND INDIVIDUAL SPECIFICITY

The body of a worm and the face of a man alike are composed of cells, organized into tissues. Yet the cells of each tissue are not generalized cells, prefabricated "modules" from which tissues are constructed. They are specialized cells adapted for specific functions in bone and muscle, in blood

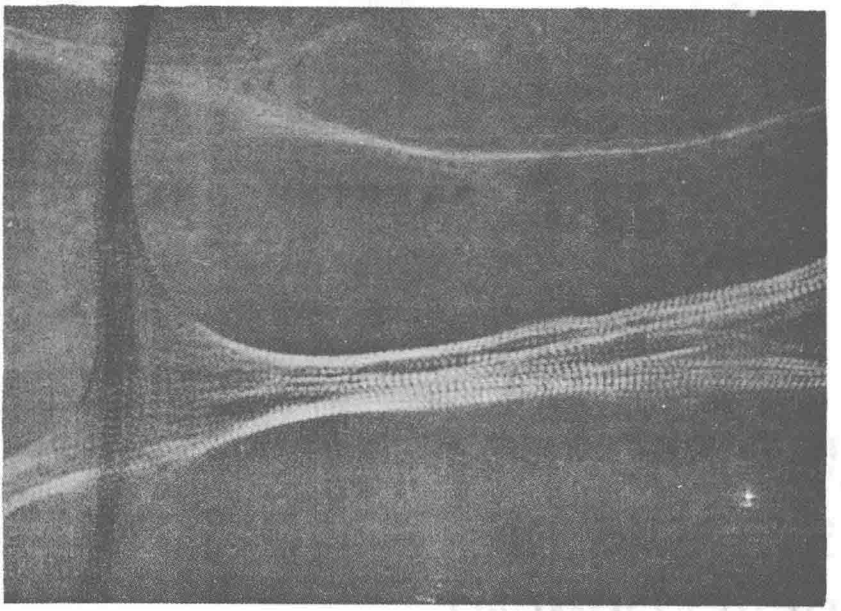

**Fig. 1-1.** Polarizing optics demonstrate cross-striated myofibrils in chick embryo leg muscle growing *in vitro*. See also Fig. 9-1. (By courtesy of I. R. Konigsberg.)

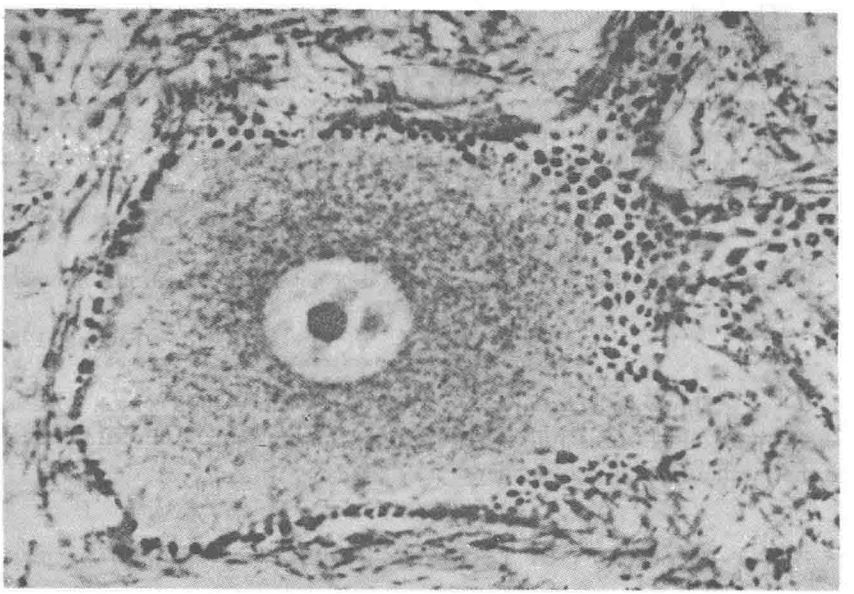

**Fig. 1-2.** Photomicrograph of the cell body of a motor neuron with many synapses on its surface. Short lengths of presynaptic axons are stained black. (From G. L. Rasmussen, *New Research Techniques of Neuroanatomy*, by courtesy of the author and Charles C Thomas, Publisher.)

and nerve. Nor have they ever been truly generalized, archetypal cells, not since the moment they were first derived, by cell division, from the fertilized egg. They have been embryonic cells, constantly changing in a changing chemical environment. From the very beginning, each cell has its characteristic specificity, which can often be traced back to a specific region of the cytoplasm in the egg in which development began. To be sure, a motor neuron has not always been a motor neuron; but what is its "birth date"? As Hamburger tells us, its development is a gradual process. Do we mark its beginning as the first subtle change in the surface of the cell, affecting its associations with its neighbors and influencing, ultimately, its placement in the body plan of the embryo? How long before it extends its first, pioneering axon is it irrevocably destined to become a neuron? See Figs. 1-1 through 1-4.

Thus the daughter cells derived from the egg follow increasingly diverse and specialized pathways resulting in the heterogeneity of cell types characteristic of the adult. Yet we know that during cell division each daughter cell receives an identical set of chromosomes, hence should be genetically identical. In this and in our earlier phrase, "a specific region of the cytoplasm" are the major clues that mark the beginning of our trail. There is no compelling reason to doubt the validity of our underlying assumption that the genes of developing cells, of muscle cells, and of neurons are identical; hence we must look beyond the gene, to regional differences in the cytoplasm of the egg. We must look to interrelations between the genes and the cytoplasm, to the cell's inner controls.

But in the embryo the kidney cells, muscle cells, and neurons do not develop, or function, in isolation. They develop as part of the whole. What properties of kidney cells determine the shape and size of the kidney? What properties of the many types of cells of which it is composed determine the shape and size of a worm—or a man? Clearly we must examine not only the cell's inner controls, but the manner in which a cell interacts with its neighbors and its microenvironment. How do cells impinge upon and influence each other?

At the end of our trail lies an even larger, and equally intriguing question, that of the biological basis of individuality.

From the fertilized human egg, about 0.14 mm in diameter, only barely visible to the naked eye (see Fig. 1-5), we emerge as individuals with combinations of behavioral, chemical, and structural properties that stamp us as unique, except for those few of us who have an identical twin. We believe that this concept of the uniqueness of the individual applies equally to man and mouse, to fish and fowl. Whether each cockroach and each jellyfish, or each oak and each linden is unique is less certain, largely because the techniques on which our ideas of individuality are based cannot yet be applied equally well to all living forms. We will not be far wrong, however, if we

**Fig. 1-3.** A longitudinal section through a small part of a striated muscle fiber. In this fiber the fibrous elements of the cytoplasm are prominently developed and collected into large bundles. The endoplasmic reticulum is crowded into the space between the myofibrils. × 50,000. (From K. R. Porter, in *The Nature of Biological Diversity*, by courtesy of the author and McGraw-Hill Book Company.)

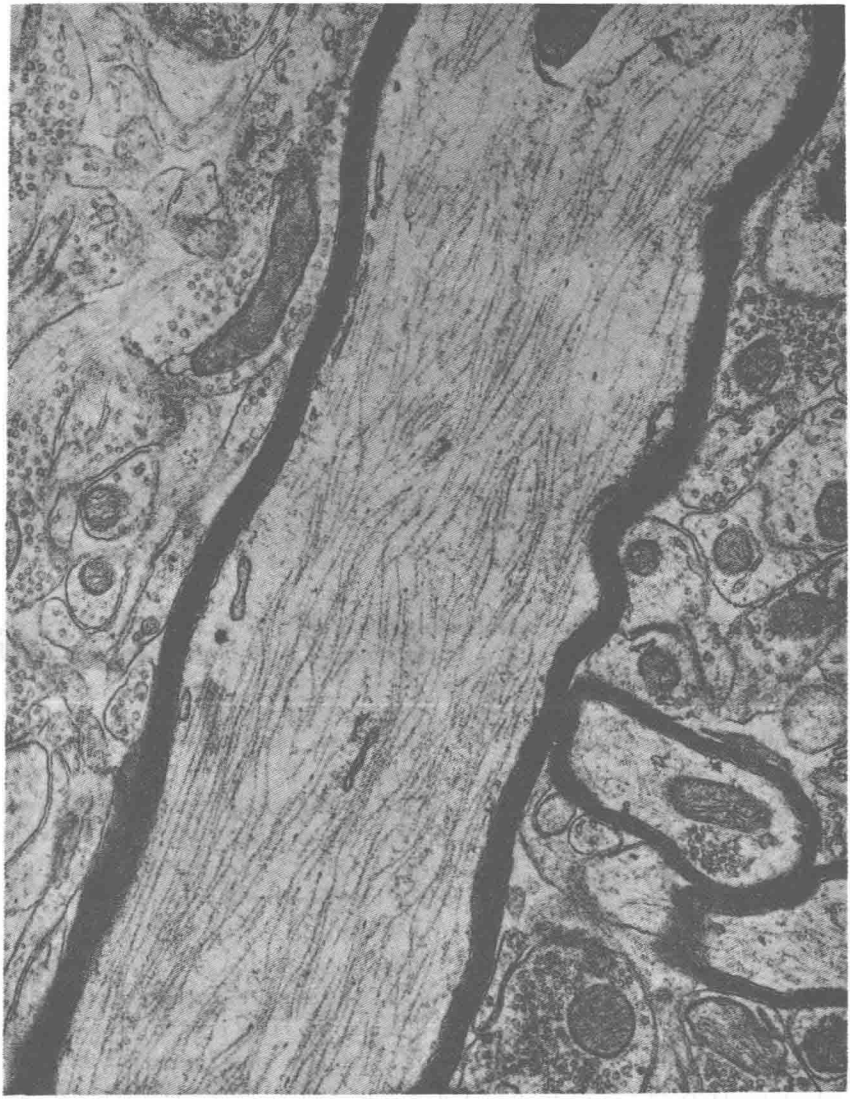

**Fig. 1-4.** A longitudinal section through a myelinated nerve fiber in the rat cerebral cortex. The axoplasm, filled with numerous long filaments, is bounded by the dense myelin sheath. (From K. R. Porter, in *The Nature of Biological Diversity*, by courtesy of the author and McGraw-Hill Book Company.)

assume at the outset that, apart from those creatures which in nature or by the artifice of the experimenter or animal or plant breeder have identical genes, each living being is unique.

Let us begin by asking, "What is the evidence for the uniqueness of the

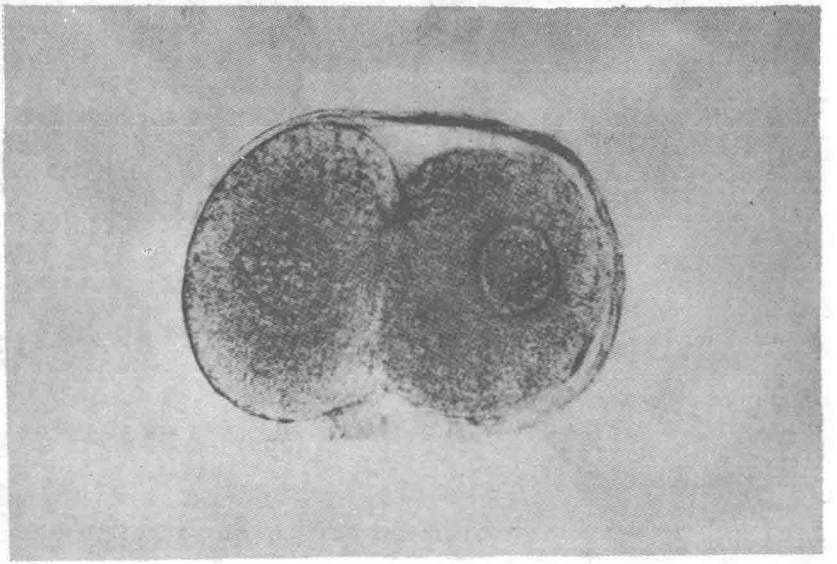

**Fig. 1-5.** Photomicrograph of 2-cell human ovum. × 500. (From A. T. Hertig, J. Rock, E. C. Adams and W. J. Mulligan, in *Contributions to Embryology*, 35, by courtesy of Carnegie Institution of Washington.)

individual?" Not all the questions we will ask about development can be answered as directly as this one. We can tell, at least in the vertebrates, whether two animals are identical or whether they are different simply on the basis of whether grafts of their skin can be exchanged successfully. The failure of *homografts*, as grafts between nonidentical members of the same species are called, leads to one of the key generalizations of our time. This fundamental argument, established by several generations of biologists and surgeons, states that the response of an animal to such a graft is an immunological reaction, related to the reaction of the animal to invasion by bacteria. We shall consider its mechanism in Chapter 12. The response is similar in all the vertebrates from fishes to man. At first a skin homograft heals; its epithelial cells begin to divide. For a time, then, such grafts behave like *autografts*, that is, grafts of the individual's own skin, transplanted from one part of his body to another, which persist for the life of the animal. But, in contrast to autografts, homografts are thrown off, following the breakdown of their blood vessels. The time required for their destruction varies in different species and, in the cold-blooded animals, also depends on the temperature; in many mammals the process is completed within 10 to 12 days.

We have already alluded to the fundamental factor determining whether a graft will be accepted or destroyed: the genetic relation between donor

and host. The "rule" is well known: if a graft carries compatibility genes (even one) that are absent in the host, it evokes an antagonistic response. The absence in the donor of such genes present in the host is not significant. The remarkable, subtle nature of these differences is shown best in certain highly inbred lines of mice: grafts made from females to males succeed, whereas those made from males to females fail. Otherwise genetically identical to the females, the males differ by containing a Y chromosome and the products of the genes contained in it.

Thus we know that the cells of each individual—not just skin cells or muscle cells or kidney cells, but all the cells of the body—contain genetically controlled specific molecules or groups of molecules that stamp it as unique. And, as we shall see, during development specific lines of cells emerge with the capacity of recognizing these subtle differences.

But why, you may ask, have we included in our introduction to development an approach that might be treated by other authors under the heading of immunology? This approach, perhaps more than any other we might select, brings into focus three concepts which underlie all developmental biology and will permeate our discussion.

First, all development rests ultimately on the genes. The fabrication of a macromolecule, the final form of an organ, or the recognition of a homograft must involve a series of interactions beyond the gene, often beyond the individual cell; but eventually it will be necessary to trace the origin of these interactions to the structure of deoxyribonucleic acid (DNA) and the control of its function. Moreover, this approach provides the necessary background for evidence, to be advanced later, that genes may be activated throughout development, and raises the question of whether they are acting in adult tissues.

Second, this approach extends our discussion beyond the interactions of molecules and to cellular interactions. Again, it will be necessary, finally, to trace these interactions to their molecular origins, but in studying the problems of development it is helpful to view them broadly at first before plunging into the inner workings of the cell.

Third, together with evidence to be presented later, this approach stresses the properties of embryos as distinct from properties of adults. Too often in studying development we think of an embryo as an adult in miniature, failing to realize that the characteristic properties of an embryo or an embryonic cell change with time. Although the metabolic requirements of a neuron in the adult brain may differ from those of a neuron in the adult peripheral nervous system, the requirements for the *formation* of either during embryonic life may differ in yet other ways. *The requirements for making a neuron may differ drastically from those for maintaining it.* The importance of recognizing the special properties of embryos is underlined if we recall that agents like the