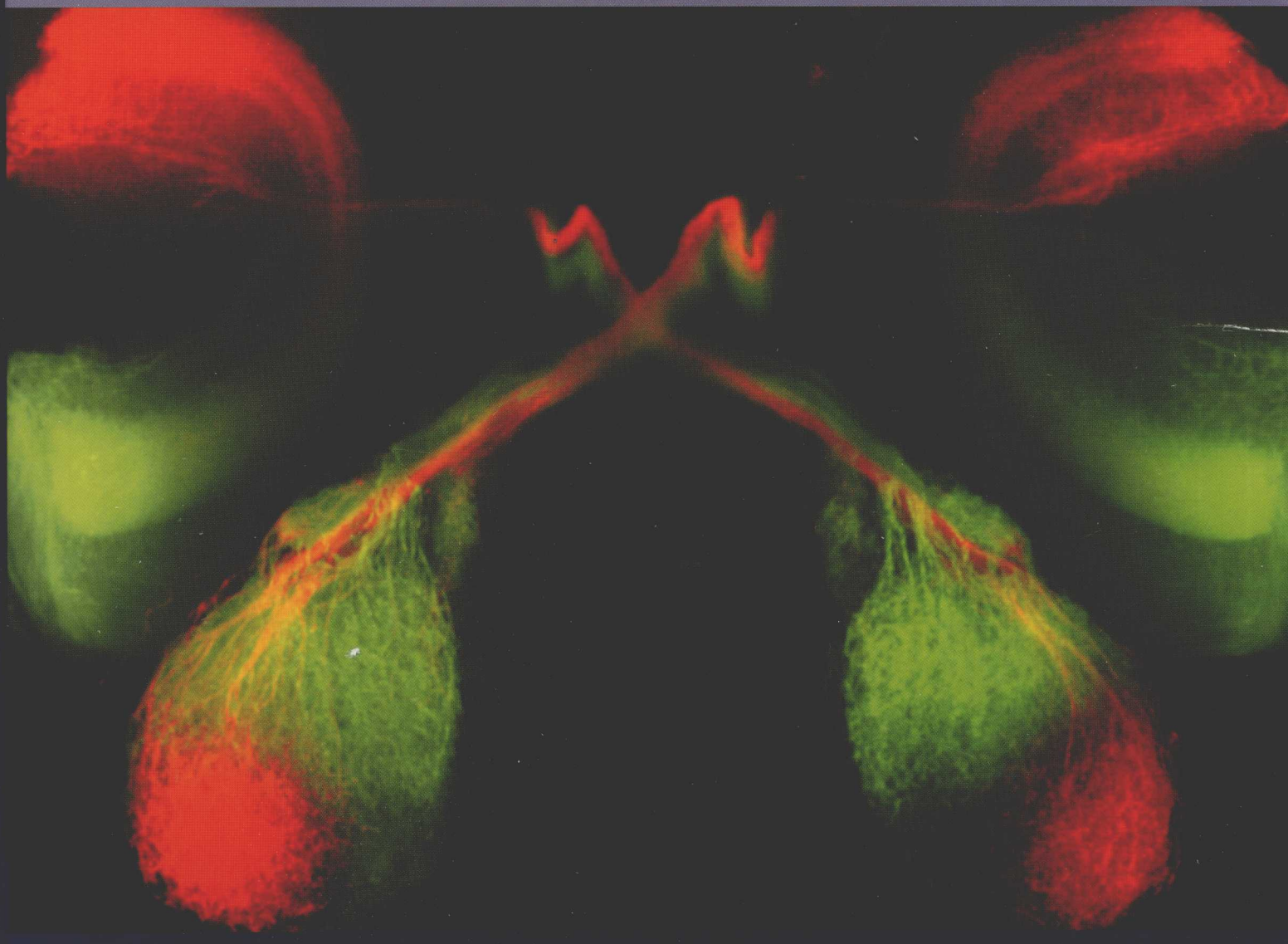
Development of the Nervous System

THIRD EDITION



Dan H. Sanes • Thomas A. Reh • William A. Harris

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DEVELOPMENT OF THE NERVOUS SYSTEM

THIRD EDITION

To our families

Preface to the Third Edition

In a field of science where the tools of investigation continue to improve dramatically and the challenge is to understand the construction of what is, arguably, the most complex object in our known universe, it is not unexpected that this third edition of ***Development of the Nervous System*** required extensive revision. Moreover, it has become increasingly clear that, in many respects, the processes of neural development continue in the "mature" adult brain. Discoveries in adult neurogenesis and plasticity have profound implications for brain function throughout life. Moreover, abnormalities in developmental mechanisms lead to brain disorders that only become manifest in adulthood. Our understanding of these developmental processes holds the promise for emerging therapies, such as deriving neurons and glia from embryonic stem cells. In this way, the study of neural development has never been more relevant.

Experts in various subfields of neural development helped us by reviewing each chapter, telling us what they thought was missing, wrong, needed updating, or should be removed from the text. They also suggested where entire sections of the book should be approached afresh, emphasizing new conceptual angles. We took most of their excellent advice. However, we were mindful that many of the older studies in our field have stood the test of time, and continue to serve as the core knowledge of neural development. This core still forms the storyline of the textbook. We hope that those of you who were content with our second edition, particularly for teaching purposes, will be comfortable with the third edition. The book is built on the same foundation, yet we have embraced ideas that have gained in acceptance and included several new studies to convey the excitement that is part of a field where very recent discoveries continue to have enormous impact. We were cautious, however, about including too much of this new material for two reasons. First, we wanted to keep the size of the book the same. Second, experience has taught us that what is new and exciting will not always turn out to be as pivotal for the field as it now appears. The future will be the best judge of which studies become classics and which studies will form the core of future textbooks.

Therefore, though we are enormously grateful to many colleagues (listed below) who have contributed advice and material to this third edition, the choice of what to include and what not to include was ours alone. We accept responsibility for any deficits in concept or coverage. Our thanks go explicitly to the following people who helped us with the third edition: Michael Bate, John Bixby, Steve Burden, Martha Constantine-Paton, Ford Ebner, Gord Fischell, John Flanagan, Francois Guillemot, Christopher Henderson, Christine Holt, Chris Kintner, Lynne Kiorpes, Alex Kolodkin, Vibhakar Kotak, Matthias Landgraf, Jeff Lichtman, Tony Movshon, Alan Roberts, John Rubenstein, Peter Scheiffele, Josh Weiner, Ed Ziff, Lance Zirpel. And a special note of thanks to the editorial staff at Elsevier: Clare Caruana, Mica Haley, Johannes Menzel, and Melissa Turner.

Dan H. Sanes
Thomas A. Reh
William A. Harris
January 2011

Preface to the Second Edition

The human brain—perhaps the most complex object in our universe—is composed of billions of cells and trillions of connections. It is truly a wonder of enormous proportions. Although we are far from a thorough understanding of our brains, study of the way that the cellular constituents of the nervous system, the neurons and glia, work to produce sensations, behaviors, and higher order mental processes has been a most productive area of science. However, more and more, neuroscientists are realizing that we are studying a moving target-growth and that changes are integral to brain function, forming the very basis for learning, perception, and performance. To comprehend brain function, then, we must understand how the circuits arise and the ways in which they are modified during maturation. Santiago Ramón y Cajal, one of the founders of modern neuroscience, was able to make his remarkable progress in studies of the cellular makeup of the nervous system in large part because of his work with the embryonic brain, choosing to study "the young wood, in the nursery stage...rather than the...impenetrable...full grown forest."

The construction of the brain is an integrated series of developmental steps, starting with the decision of a few early embryonic cells to become neural progenitors and nearing completion with the emergence of behavior, which is the scope of this book. Interactions with the world continuously update and adapt synaptic connections within the brain, and the mechanisms by which these changes occur are fundamentally a continuation of the same processes that sculpted the emerging brain during embryogenesis.

Studies of development have also led to insights about the evolutionary relationships among organisms. The dogma of phylogeny and ontogeny of the last century has been superseded by our current deeper understanding of the ways in which evolutionary change can be effected through changes in development. The brain is no exception to these rules, and we can expect that much insight into the evolution of that which makes us most human will be gained from an appreciation of how developmental processes are modified over time.

The goal of this text is to provide a contemporary overview of neural development both for undergraduate students and for those who have some background in the field of biology. This intent is not compatible with a comprehensive review of the literature. In the first edition, we noted that there were about 54,000 papers published in this field between 1966 and 1999. Another 25,000 have appeared during the past 4 years (using the search string "neural or neuron or nervous" and "development or embryology or maturation" and 2000:2004). We charted a compromise between the need to update students and our strong inclination to hold their attention. The book does not contain exhaustive lists of molecular families, and the most current review articles must serve as an appendix to our text. Since the text does not encompass many exciting areas of research, students will find themselves quickly turning to specialized texts and reviews.

Among those who helped us through discussion and editorial comment are: Chiye Aoki, Michael Bate, Carla Shatz, Ford Ebner, Edward Gruberg, Christine Holt, Lynne Kiorpes, Vibhakar Kotak, Tony Movshon, Ron Oppenheim, Sarah Pallas, Sheryl Scott, Tim Tully, and Lance Zirpel.

Finally, we acknowledge our editor, Johannes Menzel, with particular gratitude, for his help, advice, and perseverance.

Dan H. Sanes
Thomas A. Reh
William A. Harris
July 2005

Preface to the First Edition

The human brain is said to be the most complex object in our known universe, and the billions of cells and trillions of connections are truly wonders of enormous proportions. The study of the way that the cellular elements of the nervous system work to produce sensations, behaviours, and higher order mental processes has become a most productive area of science. However, neuroscientists have come to realize that they are studying a moving target: growth and change are integral to brain function and form the very basis by which we can learn anything about it. As the behavioral embryologist George Coghill pointed out, "Man is, indeed, a mechanism, but he is a mechanism which, within his limitations of life, sensitivity and growth, is creating and operating himself." To understand the brain, then, we need to understand how this mechanism arises and the ways in which it can change throughout a lifetime.

The construction of the brain is an integrated series of developmental steps, beginning with the decision of a few early embryonic cells to become neural progenitors. As connections form between nerve cells and their electrical properties emerge, the brain begins to process information and mediate behaviors. Some of the underlying circuitry is built into the nervous system during embryogenesis. However, interactions with the world continuously update and adapt the brain's functional architecture. The mechanisms by which these changes occur appear to be a continua-tion of the processes that sculpt the brain during development. Since the text covers each of these developmental steps, it is relatively broad in scope.

An understanding of the development of the nervous system has importance for biologists in a larger context. Studies of development have led to insights into the evolutionary relationships among organisms. The dogma of phylogeny and ontogeny of the last century has been superseded by a deeper understanding of the ways in which evolutionary change can be effected through changes in development. The brain is no exception to these rules. We should expect that insight into the evolution of that which makes us most human will be gained from an appreciation of how developmental processes are modified over time.

The goal of this text is to provide a contemporary overview of neural development for undergraduate students or those who have some background in the filed of biology. This intent is not compatible with a comprehensive review of the literature. A recent MEDLINE search of publications in the field of neural development [(neural or neuron or nervous) and (development or embryology or maturation)] yielded 56,840 papers published between 1966 and 1999. We admit, up front, to having read only a fraction of these papers or of the thousands that were published before 1966. As a practical matter, we made use of authoritative books, contemporary review articles, hallway conversations, and e-mail consultations to select the experiments that are covered in our text. Even so, we expect that important contributions have been missed inadvertently. Therefore, advanced students will find themselves quickly turning to specialized texts and reviews. Another compromise that comes from writing an undergraduate biology book well after the onset of the revolution in molecular biology is that all subjects now have a rather broad cast of molecular characters. In addition, the most instructive experiments on a particular class of molecules have often been performed on non-neural tissue. Even if we chose to cover only the genes and proteins whose roles have been best characterized in the nervous system, most chapters would run the risk of sounding like a (long) list of acronyms. Therefore, we

charted a compromise between the need to update students and our strong inclination to hold their attention. The book does not contain exhaustive lists of molecular families, and the most current articles must serve as an appendix to our text.

Among the many scientists who helped us through discussions, unpublished findings, or editorial comment are (in alphabetical order) Chiye Aoki, Michael Bate, Olivia Bermingham-McDonogh, John Bixby, Sarah Bottjer, Martin Chalfie, Hollis Cline, Martha Constantine-Paton, Ralph Greenspan, Voker Hartenstein, Mary Beth Hatten, Christine Holt, Darcy Kelley, Chris Kintner, Sue McConnell, Ilona Miko, Ronald Oppenheim, Thomas Parks, David Raible, Henk Roelink, Edwin Rubel, John Rubenstein, David Ryugo, Nancy Sculerati, Carla Shatz, and Tim Tully.

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Neural induction 1

Chapter 1 Outline

DEVELOPMENT AND EVOLUTION OF NEURONS

Almost as early as multicellular animals evolved, neurons have been part of their tissues. Metazoan nervous systems range in complexity from the simple nerve net of the jellyfish to the billions of specifically interconnected neuron assemblies of the human brain. Nevertheless, the neurons and nervous systems of all multicellular animals share many common features. Voltage-gated ion channels are responsible for action potentials in the neurons of Jellyfish as they are in people. Synaptic transmission between neurons in nerve nets is basically the same as that in the cerebral cortex in humans (**Figure 1.1**). This book describes the mechanisms responsible for the generation of these nervous systems, highlighting examples from a variety of organisms. Despite the great diversity in the nervous systems of various organisms, underlying principles of neural development have been maintained throughout evolution.

It is appropriate to begin a book concerned with the development of the nervous system with an evolutionary perspective. The subjects of embryology and evolution have long shared an interrelated intellectual history. One of the major currents of late-nineteenth-century biology was that a description of the stages of development would provide the key to the path of evolution of life. The phrase "ontogeny recapitulates phylogeny" was important at the start of experimental embryology (Gould, 1970). Although the careful study of embryos showed that they did not resemble the adult forms of their ancestors, it is clear that new forms are built upon the structures of biological predecessors. One aim of this book is to show how an understanding of the development of the nervous system will give us insight into its evolution. It is also wise to remember, as Dobzhansky pointed out, "nothing in biology makes sense except in the light of evolution."

EARLY EMBRYOLOGY OF METAZOANS

The development of multicellular organisms varies substantially across phyla; nevertheless, there are some common features. The cells of all metazoans are organized as layers. These layers give rise to the various organs and tissues, including the nervous system. These layers are generated from the egg cell through a series of cell divisions and their subsequent rearrangements. The eggs of animals are typically polarized, with differences in their cytoplasm from one "pole" to the other. Amphibians, for example, have an "animal pole" and a "vegetal pole" that is visible in the egg, since the vegetal pole contains the yolk, the stored nutrient material necessary for sustaining the embryo as it develops. Once fertilized by the sperm, the egg cell undergoes a series of rapid cell divisions, known as cleavages. There are many variations of cleavage patterns in embryos, but the end result is that a large collection of cells, the blastula, is generated over a relatively short period of time. In many organisms the cells of the blastula are arranged as a hollow ball, with an inner cavity known as a blastocoel. The rearrangement of this collection of cells into the primary (or germ) layers is called *gastrulation.* Gastrulation can occur via a variety of mechanisms, but all result in an inner, or endodermal, layer of cells, an outer layer of cells, the ectoderm, and a layer of cells between the two other layers, known as the mesoderm (Gilbert and Raunio, 1997). The middle layer can be derived from either the ectoderm (ectomesoderm) or the inner layer (endomesoderm). During the process of gastrulation, the cells of the mesoderm and endoderm move into the inside of the embryo, often at a single region, known as the blastopore. Once the endoderm and mesoderm are inside the ball, they usually obliterate the blastocoel and form a new cavity, the archenteron, or primitive gut. Animals can be divided in two on the basis of whether the mouth forms near the point of this blastopore (in protostomes) or at a distant site (in deuterostomes). Once these three primary germ layers are established, the development of the nervous system begins. A more detailed description of the development of the other organ systems is beyond the scope of this text. Nevertheless, one should keep in mind that the development of the nervous system does

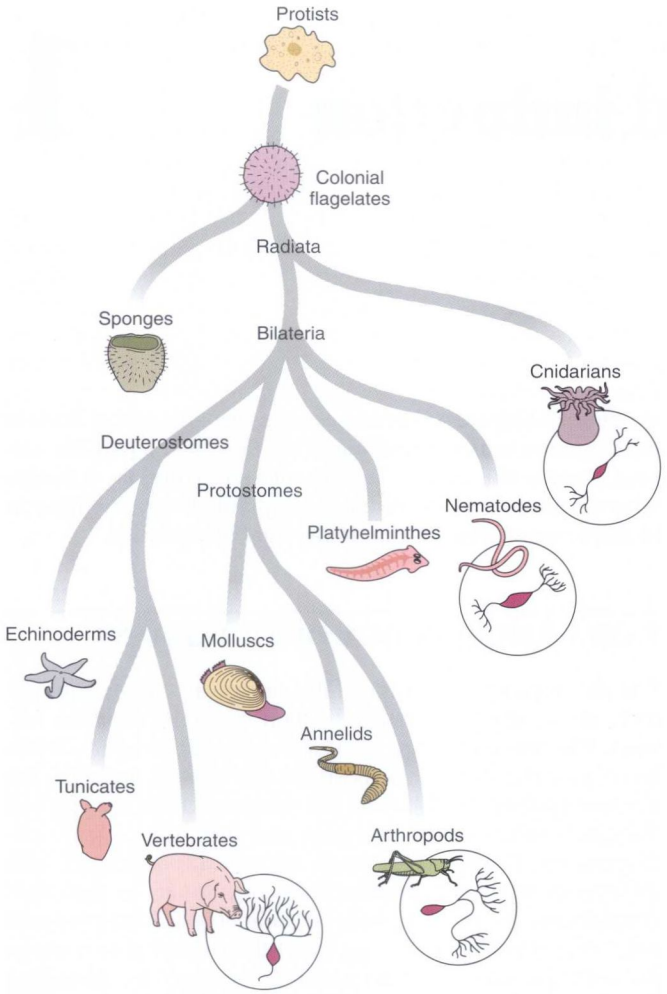

Fig. 1.1 Neurons throughout the evolution of multicellular organisms have had many features in common. All animals other than colonial flagellates and sponges have recognizable neurons that are electrically excitable and have long processes. The Cnidarians have nerve networks with electrical synapses, but synaptic transmission between neurons is also very ancient.

not take place in a vacuum, but is an integral and highly integrated part of the development of the animal as a whole.

The next three sections will deal with the embryology of several examples of metazoan development: nematode worms (*Caenorhabditis elegans*); insects (*Drosophila melanogaster*); and several vertebrates (frogs, fish, birds, and mammals). The development of these animals is described because they have been particularly well studied for historical and practical reasons. However, one should take these examples as representative, not as definitive. The necessity of studying many diverse species has become critical to the understanding of the development of any one species.

DERIVATION OF NEURAL TISSUE

The development of the nervous system begins with the segregation of neural and glial cells from other types of tissues. The many differences in gene expression between neurons and muscle tissue, for example, arise through the progressive narrowing of the potential fates available to a blast cell during development. The divergence of neural and glial cells from other tissues can occur in many different ways and at many different points in the development of an organism. However, the cellular and molecular mechanisms that are responsible for the divergence of the neural and glial lineages from other tissues are remarkably conserved.

C. ELEGANS

The development of *C. elegans*, a nematode worm, highlights the shared lineage of the epidermal and neural cell fates. These animals have been studied primarily because of their simple structure (containing only about a thousand cells), their rapid generation time (allowing for rapid screening of new genetic mutants), and their transparency (enabling lineage relationships of the cells to be established). These nematodes have a rigid cuticle that is made of collagenous proteins secreted by the underlying cells of the hypodermis. The hypodermis is analogous to the epidermis of other animals, except that it is composed of a syncytium of nuclei rather than of individual cells. They have a simple nervous system, composed of only 302 neurons and 56 glial cells. These neurons are organized into nerve cords. The nerve cords are primarily in the dorsal and ventral sides of the animals, but there are some neurons that run along the lateral sides of the animal as well. The nematodes move by a series of longitudinal muscles, and they have a simple digestive system.

Nematodes have long been a subject for developmental biologists' attention. Theodore Boveri studied nematode embryology and first described the highly reproducible pattern of cell divisions in these animals in the late 1800s. Boveri's most famous student, Hans Spemann, whose work on amphibian neural induction will be described below, worked on nematodes for his Ph.D. research. The modern interest in nematodes, however, was motivated by Sydney Brenner, a molecular biologist who was searching for an animal that would allow the techniques of molecular genetics to be applied to the development of metazoans (Brenner, 1974).

Because of the stereotypy in the pattern of cell divisions, the lineage relationships of all the cells of *C. elegans* have been determined (Sulston et al., 1983). The first cleavage produces a large somatic cell, the AB blastomere, which gives rise to most of the hypodermis and the nervous system and the smaller germline P cell, which in addition to the gonads will also generate the gut and most of the muscles of the animal (**Figure 1.2**). Subsequent cleavages produce the germ cell precursor, P4, and the precursor's cells for the rest of the animal: the MS, E, C, and D blastomeres (Figure 1.2), and these cells all migrate into the interior of the embryo, while the AB-derived cells spread out over the outside of the embryo completing gastrulation (**Figure 1.3**). The next phase of development is characterized by many cell divisions and is known as the *proliferation phase*. Then an indentation forms at the ventral side of the animal marking the beginning of the morphogenesis stage, and as this indentation progresses, the worm begins to take shape (Figure 1.3). At this point, the worm has only 556 cells and will add the remaining cells (to the total of 959) over the four larval molts. The entire development of the animal takes about two days.

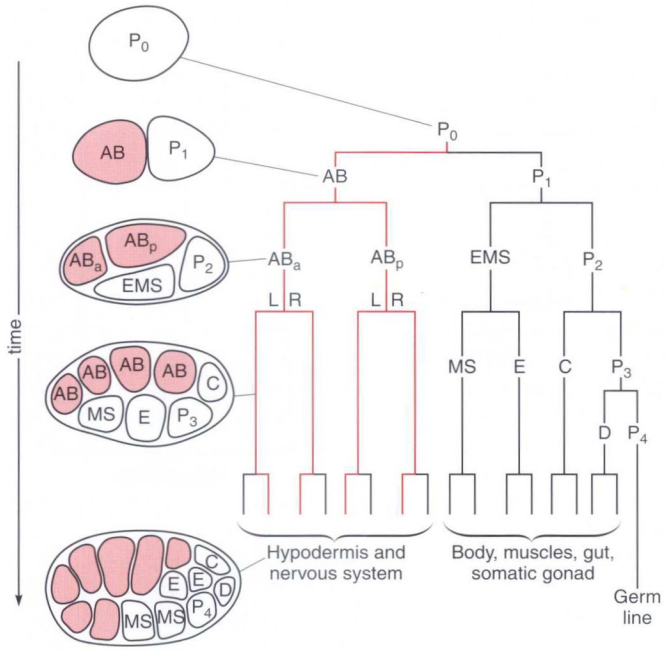

Fig. 1.2 The nervous system shares a common cellular lineage with the ectoderm. The cell divisions that generate the *C. elegans* nematode worm are highly reproducible from animal to animal. The first division produces the AB blastomere and the P1 blastomere. The germ line is segregated into the P4 blastomere within a few divisions after fertilization. The subsequent divisions of the AB blastomere go on to give rise to most of the neurons of the animal, as well as to the cells that produce the hypodermis—the epidermis of the animal.

The neurons of *C. elegans* arise primarily from the AB blastomere, in lineages shared with the ectodermally derived hypodermis. An example of one of these lineages is shown in Figure 1.3. The Abarpa blastomere can be readily identified in the 100-min embryo through its position and lineal history. This cell then goes on to give rise to 20 additional cells, including 9 neurons of the ring ganglion. The progeny of the Abarpa blastomere, like most of the progeny of the AB lineage, lie primarily on the surface of the embryo prior to 200 min of development. At this time, the cells on the ventral and lateral sides of the embryo move inside and become the nervous system, whereas the AB progeny that remain on the surface spread out to form the hypodermis, a syncytial covering of the animal. Most of the neurons arise in this way; of the 222 neurons in the newly hatched *C. elegans*, 214 arise from the AB lineage, whereas 6 are derived from the MS blastomere and 2 from the C blastomere.

DROSOPHILA

The development of the fruit fly, *Drosophila*, is characteristic of many arthropods. Unlike the embryos of the nematode, where cleavage of the cells occurs at the same time as nuclear divisions, the initial rounds of nuclear division in the *Drosophila* embryo are not accompanied by corresponding cell divisions. Instead, the nuclei remain in a syncytium up until just prior to gastrulation, three hours after fertilization. Prior to this time, the dividing nuclei lie in the interior of the egg, but they then move out toward the surface and a process known as cellularization occurs, and the nuclei are surrounded by plasma membranes. At this point the embryo is known as a cellular blastoderm.

The major part of the nervous system of *Drosophila* arises from cells in the ventrolateral part of the cellular blastoderm (**Figure 1.4**, top). Soon after cellularization, the ventral furrow, which marks the beginning of gastrulation, begins to form (Figure 1.4, middle). At the ventral furrow, cells of the future mesoderm fold into the interior of the embryo. The process of invagination occurs over several hours, and the invaginating cells will continue to divide and eventually will give rise to the mesodermal tissues of the animal. As the mesodermal cells invaginate into the embryo, the neurogenic region moves from the ventrolateral position to the most ventral region of the animal (Figure 1.4). The closing of the ventral furrow creates the ventral midline, a future site of neurogenesis. On either side of the ventral midline is the neurogenic ectoderm, tissue that will give rise to the ventral nerve cord, otherwise known as the central nervous system (**Figure 1.5**). A continuation of the neurogenic region into the anterior of the embryo, is called the procephalic neurogenic region and gives rise to the cerebral ganglia or brain.

Drosophila neurogenesis then begins in the neurogenic region; some cells enlarge and begin to move from the outside layer into the inside of the embryo (Figure 1.5). At the beginning of neurogenesis, the neurogenic region is a single cell layer; the first morphological sign of neurogenesis is that a number of cells within the epithelium begin to increase in size. These larger cells then undergo a shape change and squeeze out of the epithelium. This process is called *delamination* and is shown in more detail in Figure 1.5. The cells that delaminate are called *neuroblasts* and are the progenitors that will generate the nervous system. In the next phase of neurogenesis, each neuroblast divides to generate many progeny, known as *ganglion mother cells* (GMCs). Each GMC then generates a pair of neurons or glia. In this way, the entire central nervous system of the larval *Drosophila* is generated. However, the *Drosophila* nervous system is not finished in the larva, but rather additional neurogenesis occurs during metamorphosis. Sensory organs, like the eyes, are generated from small collections of cells in the larva (called imaginal discs) that undergo a tremendous amount of proliferation during metamorphosis to generate most of what we recognize as an adult fly.

VERTEBRATES

All vertebrate embryos develop in a fundamentally similar way, though at first appearance they seem to be quite different. In this section we will review the development of several different vertebrates: amphibians, fish, birds, and mammals. In all of these animals, multiple cleavage divisions generate a large number of cells from the fertilized oocyte. However, while gastrulation in all of these animals is basically conserved, the details of the cellular movements during this phase can look quite different.

Amphibian eggs are like those of many animals in that the egg has a distinct polarity with a nutrient-rich yolk concentrated at the "vegetal" hemisphere and a relatively yolk-free "animal" hemisphere. After fertilization, a series of rapid cell divisions, known as cleavages, divides the fertilized egg into blastomeres. The cleavage divisions proceed

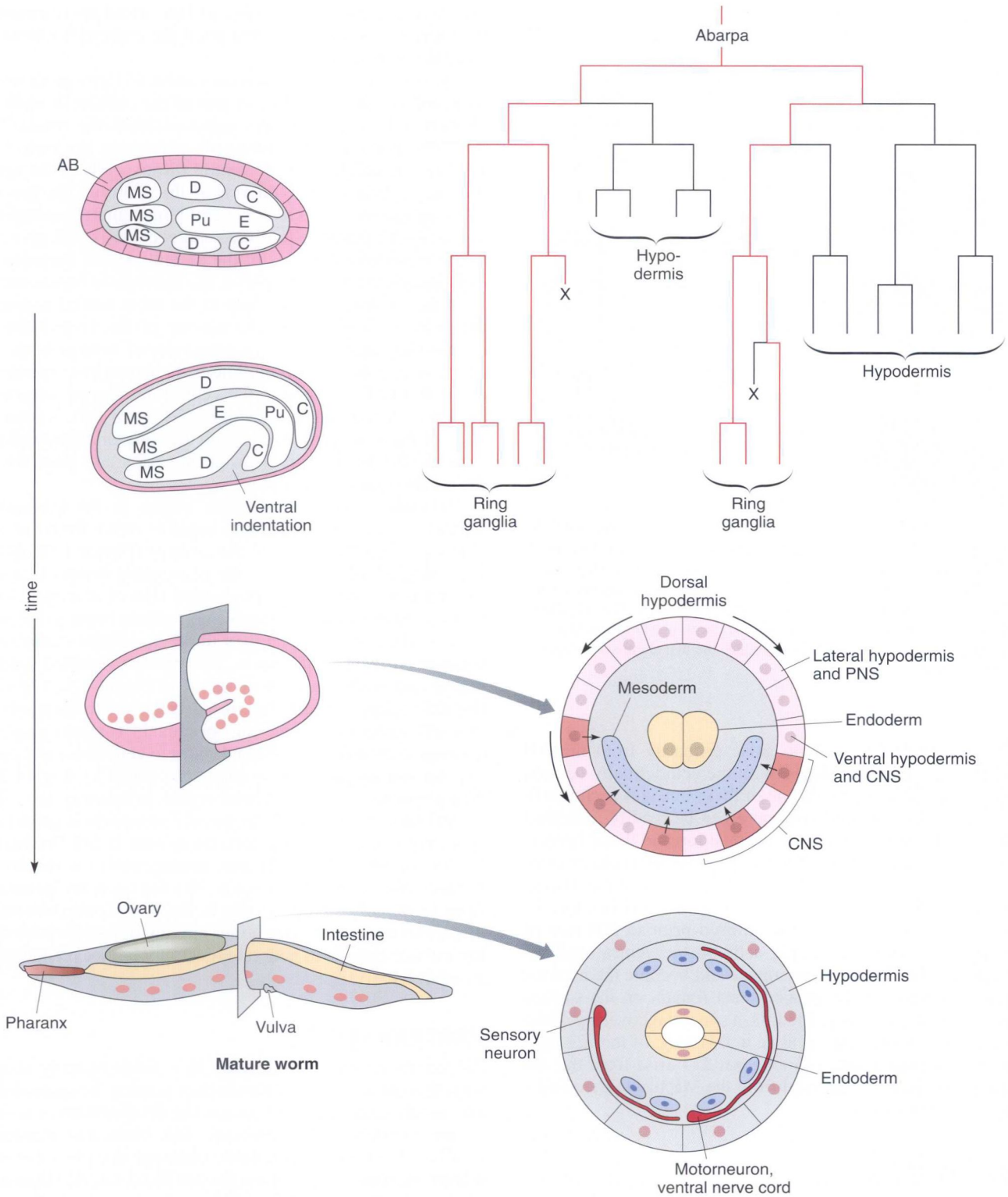

Fig. 1.3 The next phase of development of the *C. elegans* worm also highlights the shared lineages of hypodermis and neurons. During gastrulation, the MS, E, C, and D blastomeres all migrate into the interior of the embryo, while the progeny of the AB blastomeres spread out over the external surface. Once the embryo starts to take form, sections through the embryo show the relationships of the cells. The neurons are primarily derived from the ventrolateral surface, through the divisions of the AB progeny cells. As these cells are generated, they migrate into the interior and form the nerve rings. A typical lineage is also shown. The Abarpa blastomeres undergo five rounds of division, to generate 9 neurons and 10 hypodermal cells. Neural lineages are shown in red.

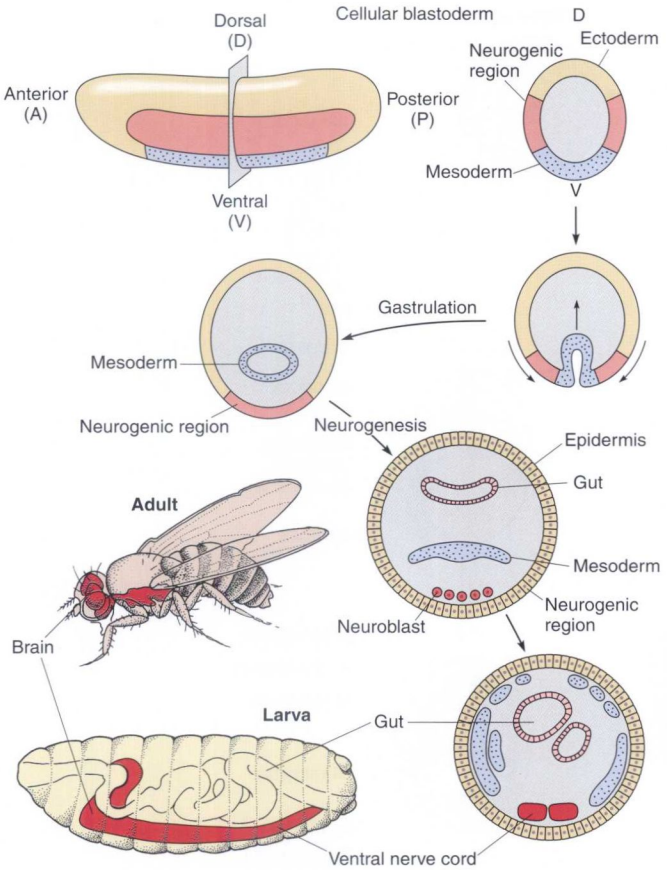

Fig. 1.4 The nervous system of the *Drosophila* is derived from the ventrolateral region of the ectoderm. The embryo is first (top) shown at the blastoderm stage, just prior to gastrulation. The region fated to give rise to the nervous system lies on the ventral-lateral surface of the embryo (red). The involution of the mesoderm at the ventral surface brings the neurogenic region closer to the midline. Scattered cells within this region of the ectoderm then enlarge, migrate into the interior of the embryo, and divide several more times to make neurons and glia. These neurons and glia then condense into the ganglia of the mature ventral nerve cord (or CNS) in the larva and the adult.

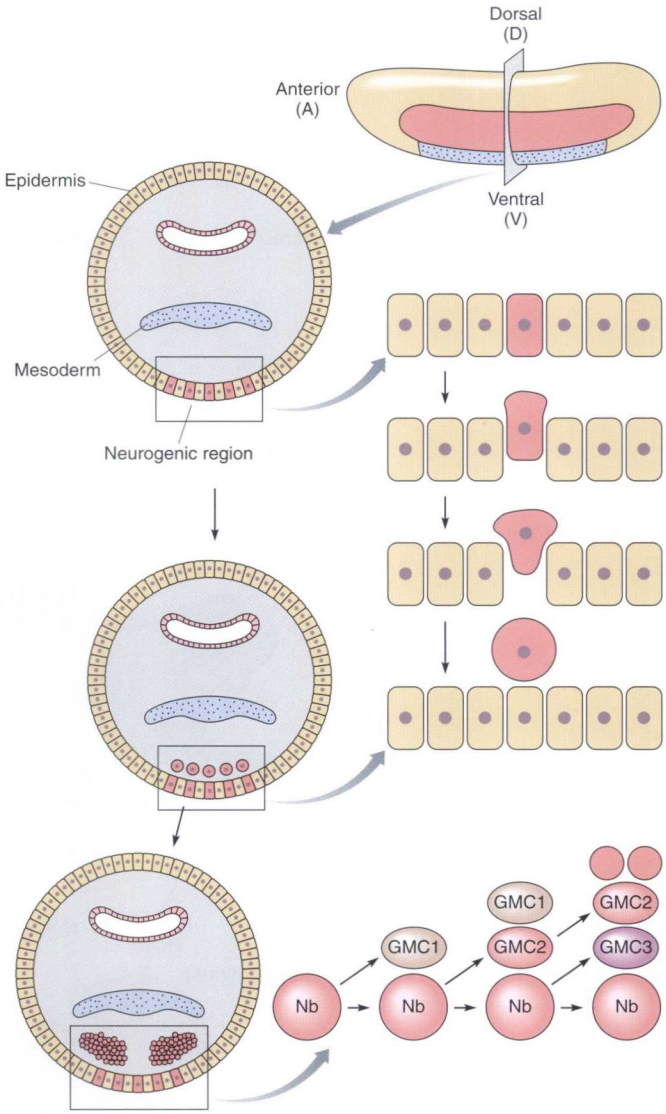

Fig. 1.5 The neuroblasts of the *Drosophila* separate from the ectoderm by a process known as delamination. The neuroblasts enlarge relative to the surrounding cells and squeeze out of the epithelium. The process occurs in several waves; after the first set of neuroblasts has delaminated from the ectoderm, a second set of cells in the ectoderm begins to enlarge and also delaminates. The delaminating neuroblasts then go on to generate several neurons through a stereotypic pattern of asymmetric cell divisions. The first cell division of the neuroblast produces a daughter cell known as the ganglion mother cell, or GMC. The first ganglion mother cell divides to form neurons, while the neuroblast is dividing again to make another GMC. In the figure, the same neuroblast is labeled through its successive stages as Nb, while the GMCs are numbered successively as they arise.

less rapidly through the vegetal hemisphere, and by the time the embryo reaches 128 cells, the cells in the animal half are much smaller than those of the vegetal half (**Figure 1.6**). The embryo is called a *blastula* at this stage. The process by which the relatively simple blastula is transformed into the more complex, three-layered organization shared by most animals is called *gastrulation.* During this phase of development, cells on the surface of the embryo move actively into the center of the blastula. The point of initiation of gastrulation is identified on the embryo as a small invagination of the otherwise smooth surface of the blastula, and this is called the *blastopore* (Figure 1.6). In amphibians the first cells to invaginate occur at the dorsal side of the blastopore (Figure 1.6), opposite to the point of sperm entry. The dorsal side of the blastopore has a special significance for the process of neural induction and much more will be said about this in this chapter.

The involuting cells lead a large number of cells that were originally on the surface of the embryo to the interior (Figure 1.6). The part of the blastula that will ultimately reside in the interior of the embryo is called the *involuting marginal zone* (IMZ). Most of these cells will give rise to mesodermally derived tissues, like muscle and bone. The first cells to involute crawl the farthest and produce the mesoderm of the anterior part of the animal (i.e., the head). The later involuting IMZ cells produce the mesoderm of more posterior regions, including the tail of the tadpole. At this point in development, the neural plate of the vertebrate embryo still largely resembles the rest of the surface ectoderm. However, shortly after its formation, the neural plate begins to fold onto itself to form a tubelike structure, the