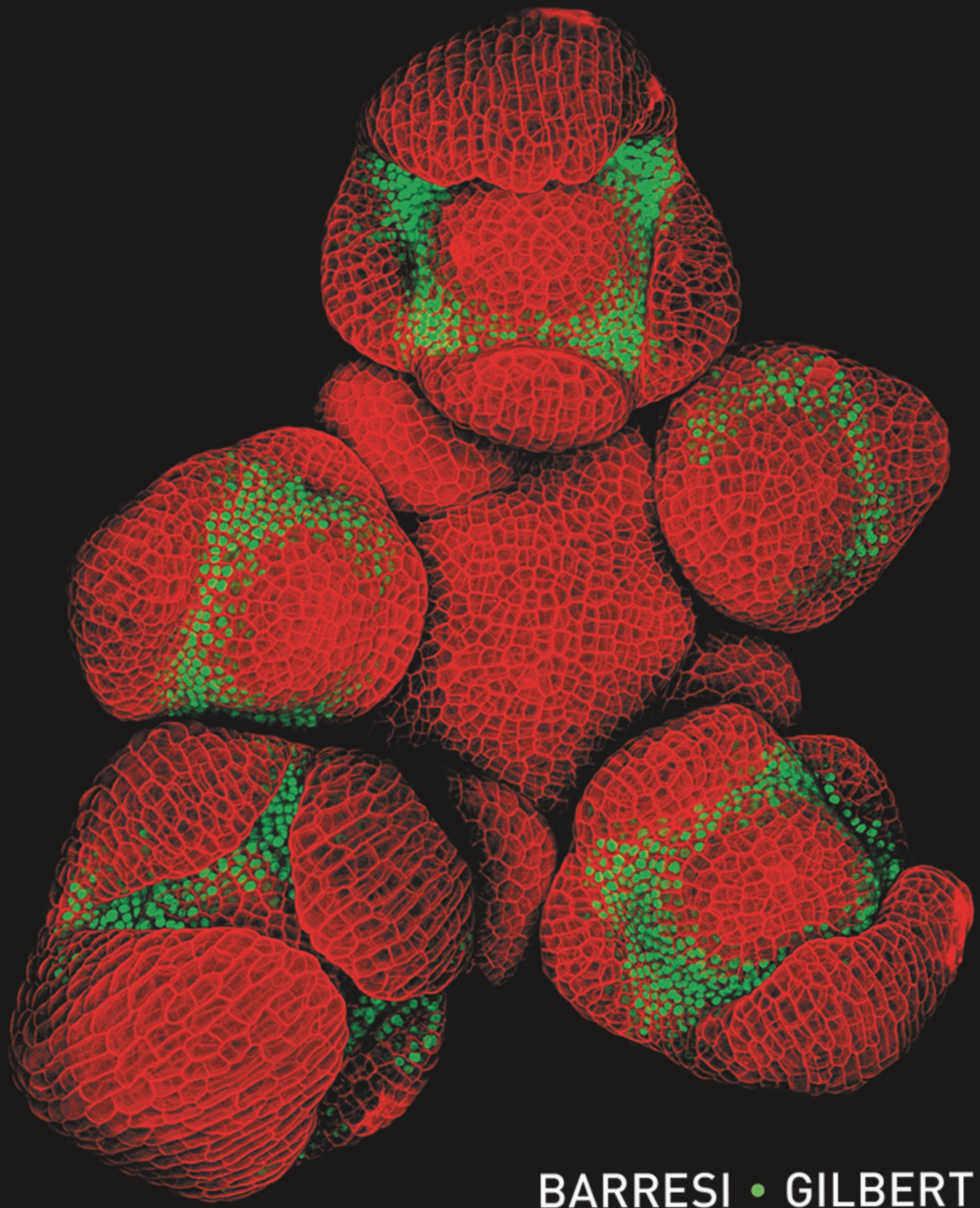
Developmental Biology

TWELFTH EDITION



BARRESI • GILBERT

Developmental Biology

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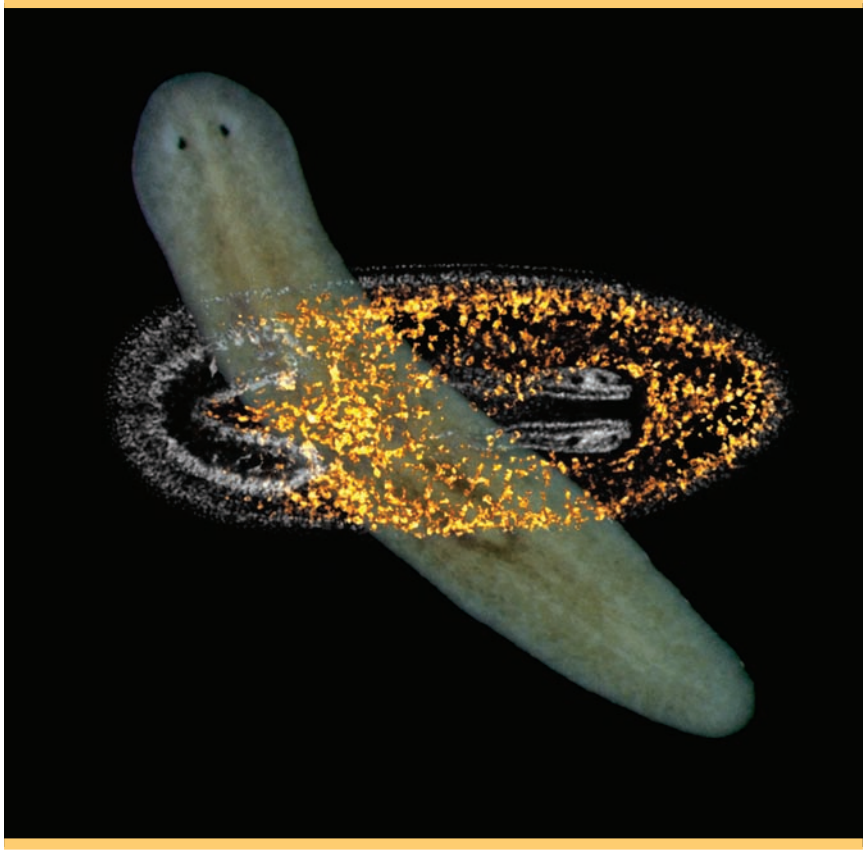

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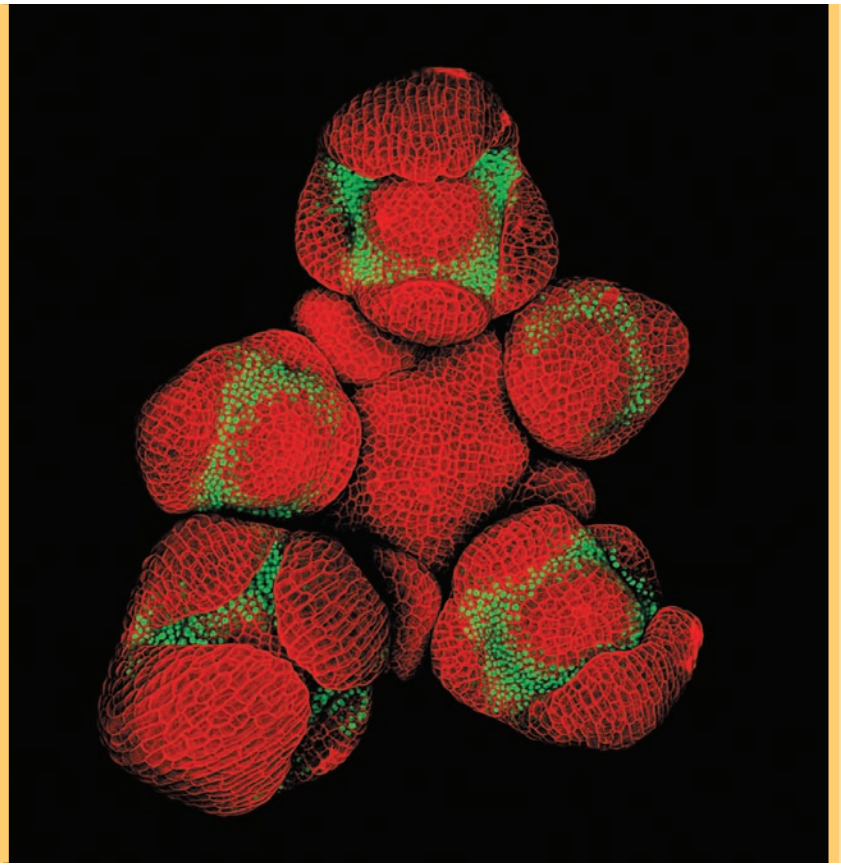

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About the Cover

What do you think the cover image looks like? Some kind of animal embryo or perhaps a miniature bear cub? Maybe even some sort of baby "Power Ranger"? Despite resembling an animal, the image shows living, developing flower buds. This 12th Edition marks the long-awaited return of plant development to *Developmental Biology*. That's right, the cover shows part of a plant—a part that nevertheless exemplifies many of the core topics emphasized in this edition. The floral meristems in the image illustrate the totipotency of plant stem cells during both embryonic development and post-embryonic regeneration. The image also shows differential expression of the *APETALA3* gene (revealed by a fluorescent reporter, green), which is confined to a ring of differentiating cells around each floral bud. (The red fluorescence comes from staining of the plant cell walls.) Aside from being visually captivating, our cover image illustrates embryogenic mechanisms used by both animals and plants, which mirrors how we have integrated plant and animal concepts in this textbook. We hope this image also makes you pause and contemplate what aspect of development it might represent—a type of self-guided investigation we encourage throughout this new edition.

Image generously provided by Dr. Nathanaël Prunet of UCLA and originally published in N. Prunet et al. 2016. Live confocal imaging of Arabidopsis flower buds. *Dev Biol* 419: 114–120.

Developmental Biology, 12th edition
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To those whose lives were most directly impacted
over the course of its creation.

My parents, Joseph and Geraldine Barresi –
Thanks for always putting your kids first.

My family, Heather, Samuel, Jonah, Luca, and Mateo –
This book was only accomplished with
your unwavering understanding, love and support.

-M. J. F. B.

To Anne, Daniel, Sarah, David, and Natalia,
whose support and humor have sustained me, and
to Alina who was born since the last edition.

-S. F. G.

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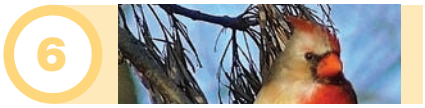

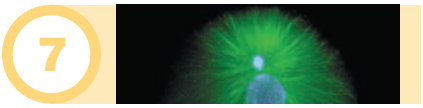

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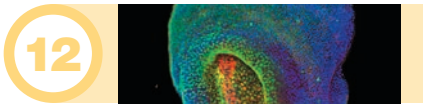

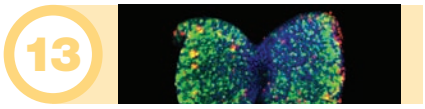

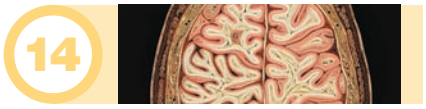

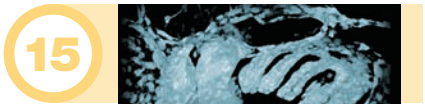

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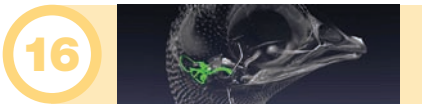

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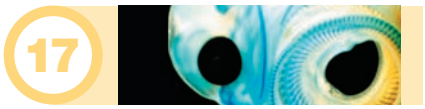

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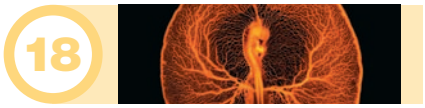

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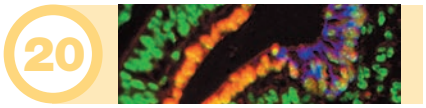

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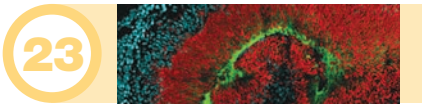

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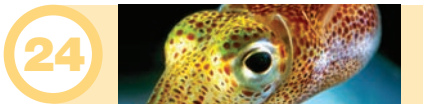

Preface: Thinking Grandly about Developmental Biology

With biology going into smaller and smaller realms, it is sometimes good to contemplate the grand scheme of things rather than the details, to "seat thyself sultanically among the moons of Saturn" (in Herman Melville's phrase). It is good, for instance, to get a perspective of developmental biology from outside the discipline rather than from inside it.

Remembering the Field's Interdisciplinary Foundations

Developmental biology, history tells us, is an interdisciplinary field that is at the foundations of biology. Indeed, before the word biology came to be used, the living world was characterized as that part of the world that was developing. The organizers of the first meeting (in 1939) of the Growth Society, which was the precursor of the Society for Developmental Biology, claimed that development must be studied by combining the insights of numerous disciplines, including genetics, endocrinology, biochemistry, physiology, embryology, cytology, biophysics, mathematics, and even philosophy. Developmental biology was to be more than embryology. It also included stem cells, which were known to generate the adult blood, and regeneration, which was seen to be the re-activation of developmental processes and which was critical for healing in vertebrates and for reproduction of hydra, flatworms, and numerous other invertebrates. The first articles published in the journal *Developmental Biology* showcased embryology, regeneration, and stem cells, and the different ways of studying them.

Throughout this new 12th edition you will see a return to some of these founding ideas of interdisciplinary developmental biology, namely regeneration, morphomechanics, plants, and the genetic control of development.

Indeed, *regeneration* has historically been a major part of developmental biology, for it is a developmental phenomenon that can be readily studied. Experimental biology was born in the efforts of eighteenth-century naturalists to document regeneration and to examine how it was possible. The regeneration experiments of Tremblay (hydras), Réaumur (crustaceans), and Spallanzani (salamanders) set the standard for experimental biology and for the intelligent discussion of one's data.

More than two centuries later, we are beginning to find answers to the great problems of both embryology and regeneration. Indeed, the conclusions of one support the research of the other. We may soon be able to alter the human body so as to permit our own limbs, nerves, and organs to regenerate. Severed limbs could be restored, diseased organs could be removed and regrown, and nerve cells altered by age, disease, or trauma could once again function normally. The ethical issues this would exacerbate are only beginning to be appreciated. But if we are to have such abilities, we first have to understand how regeneration occurs in those species that have this ability. Our new knowledge of the roles that paracrine factors and physical factors play in embryonic organ formation, plus recent studies of stem cells and their niches, has propelled what Susan Bryant has called "a regeneration renaissance." Since "renaissance" literally means "rebirth," and since regeneration can be seen as a return to the embryonic state, the term is apt in many ways.

Notice that *biophysics* was also an early part of the mix of developmental biology. This area, too, is having a renaissance. The physical connections between cells, the strength of their bonding, and the tensile strength of the material substrates of the cells are all seen to be critical for normal development. Physical forces are necessary for sperm-egg binding, gastrulation, heart development, gut development, the branching of the kidney and lung epithelia, and even the development of tumors. Physical forces can direct the development of stem cells toward particular fates, and they can determine which part of the body is left and which is right. The patella of our kneecap doesn't form until we put pressure on it by walking. In many cases, physical forces can direct gene expression. Lev Beloussov, a pioneer in this area, has called this the "morphomechanics of development."

Another area that was prominently represented in the early programs of developmental biology was *plant development*. Plant development had much in common with regeneration, as "adult" plants could redevelop entire parts of their bodies. Whereas in animal biology the study of development diverged from the study of physiology, that separation was not evident in plant biology. Moreover, while many animals quickly set aside a germline that was to become the sperm or eggs, this was not the case in plants. Such comparisons between plants and animals are now present throughout this text, and they serve to highlight the fundamental developmental processes that are present across phyla and even kingdoms of life.

But the genes remain the center of focus in developmental biology. And the more we learn about them, the more interesting and complex these genes become. New advances in "single cell transcriptomics" have given us an amazing privilege—the ability to look at the gene expression patterns of individual cells as they develop. An individual's cells may all have the same genes, but their different positions in the embryo cause different genes to be active in each cell. It's a symphony of relationships, each cell providing the context for another. If development is the performance, then the genome is the script or score. As anyone who has gone to concerts knows, different bands perform the same score differently, and the same band will play the same song differently on two successive nights. Environment is also critical—hence, the new interest in plasticity and symbiosis in development.

Developmental biology has also taken on a new role in science. More than any other biological science, it demonstrates the critical importance of processes as opposed to entities. In many organisms, the same process can be done by different molecules. "It's the song, not the singer," say Doolittle and Booth, and we can be thankful that there are redundant pathways in development—if one pathway fails, another is often able to take over its function. The entity/process split in developmental biology mirrors the particle/wave dichotomy in physics. It is a "both, and" situation, rather than an "either/or" situation. In 1908, the Scottish physiologist J. S. Haldane said, "That a meeting point between biology and physical science may at some time be found, there is no doubting. But we may confidently predict that if that meeting-point is found, and one of the two sciences is swallowed up, that one will not be biology." Developmental biology may well solve the longstanding mysteries of physics.

New to the Twelfth Edition

In this current volume, we have attempted to track this amazing fulfillment of the early promises of developmental biology. To this end, the book has undergone its own morphogenesis.

Plant development covered throughout

We have now incorporated plant material into the relevant chapters. Instead of segregating plant developmental biology into a single (and often unassigned)

chapter, we have integrated essential plant biology into the chapters on cell specification, gene regulation, cell communication, gamete production, fertilization, axis determination, organ formation, and regeneration.

Upgraded and expanded chapter on regeneration

We have also expanded the chapter on regeneration, which we are proud to say offers a unique summary of the field. It both captures the fascinating problems of post-embryonic development that regeneration seems to solve and provides a logical framework for the known mechanisms of regeneration, based on an organism's degree of regenerative capacity. We feel that this chapter will be an excellent place for anyone interested in this area to start.

Updates throughout all chapters

All of the chapters have received important updates, from the introductory chapter's broader evolutionary perspective to new material on the morphomechanics of development during *Drosophila* gastrulation and the formation of mammalian lungs. Special consideration was also given to the increasing use of whole-genome, transcriptomic approaches, which are dramatically shaping our understanding of cell differentiation.

A new, student-centered approach

From a pedagogical standpoint, it is also good to get an outside perspective of how students are learning developmental biology—*the perspective of the student experience*. For decades, it has been the responsibility of textbooks like ours to be the most comprehensive sources for the field's foundational content. Although this responsibility still remains, the reality is that students are inundated with an overwhelming myriad of sources vying for their attention. If there was ever a time a student of developmental biology needed a *guidebook* to navigate through this dense and diverse ecosystem of texts, online resources, and infinitely expanding scientific literature, the time is now and the guidebook this new volume of *Developmental Biology*.

- *Focused and streamlined coverage.* Over the years, as new knowledge has grown, so has our own textbook, which was reaching a size that might itself trigger student overload and defeat the purposes of engagement and deep learning. The information bombarding students is not going away; therefore, they need not only access to the information but also a clear guide that fosters movement from the essential ideas to the complex mechanisms and finally to inclusive invitations that welcome their research in this field. We have both reduced and reorganized the content in each chapter to achieve a clear and supportive lattice so that both the professor and the student can more easily navigate the increasing volume and complexity of developmental biology.
- *Innovative pedagogy: Empowering students to craft their own learning.* The first material students will encounter in each section of a chapter represents the most essential content. We have introduced a new element called "Further Development," which highlights content we feel represents some of the more complex ideas in the field. In addition, students will also come across invitations to view some Further Developments online. These online topics represent fantastic opportunities for students to *further develop* their understanding of developmental biology along paths of their own interest—paths of investigation that professors can have confidence match the standards of quality seen throughout the textbook (unlike some other online sources). The special in-text features of previous editions—Dev Tutorials, Developing Questions, Next Step Investigations, and citations throughout—are still in place to play

important roles in empowering students to take that final leap to engage with the developmental biology literature. To better support students' use of the research literature, we now include a new Appendix focused on how to find and analyze research articles in developmental biology.

Thanks to this new organization of content, professors and students will now be in complete control of what level of material may be most appropriate. We are proud to introduce *Developmental Biology* 12e, as it still provides direct access to all levels of the content but without diluting its quality and the overall learning experience.

Acknowledgments

First, the two authors gratefully acknowledge their mutual respect for one another and for the enjoyment of each other's work. Michael wants the community to know that Scott has been most accepting and welcoming to new ideas and that his enthusiasm for producing the best product has not wavered any day of any edition. Scott wants the community to know that he is thrilled with the new ideas that Michael has brought to the book and that Michael's commitment to undergraduate education is second to none.

Second, we are thrilled to acknowledge the importance of Mary Stott Tyler to this book. The winner of the Viktor Hamburger Education Award and the author of *Fly Cycle*, *Differential Expressions*, *The Developmental Biology Vade Mecum*, and *Inquiry Biology*, Mary has been a mixture of author, editor, and curator of contents for this 12th edition, helping us decide "what to leave in/what to leave out." As we added plant studies to the book and had to remove other studies, Mary's insight and vision for the finished book was essential.

If science is like a balloon expanding into the unknown—and the larger the balloon, the more points in contact with the unknown—then developmental biology has contacted an astounding number of unknowns. The accuracy and coverage of the 12th edition owes much to the work of the many expert reviewers who took the time to provide respectful formal and informal feedback throughout the process (see list). The organization of these reviews was consistently executed by Lauren Cahillane, Nina Rodriguez-Marty and Katie Tunkavige—thank you for making this important part possible. This 12th edition is particularly unique as it marks the new incorporation of plant developmental biology. There were numerous reviewers who offered their expertise in select chapters, thank you to all. Special thanks, however, go to Anna Edlund and Marta Laskowski for their reviews of the plant content. They were very patient with us, and any misunderstandings are those of the authors.

This edition also marks a dramatic change to the publishing of *Developmental Biology*. With the retirement of Andy Sinauer, Sinauer Associates has become an imprint of Oxford University Press. Our book overlaps these two periods, and has seen the change of managers, art directors, and our long-time editor. We thank both Sinauer Associates and Oxford University Press for their great efforts in sustaining the book during this period of metamorphosis. We wish to especially thank Dean Scudder for taking on the managerial tasks and allowing us to work on new models of science education during this transition. Moreover, half-way through production of this edition, Jason Noe of Oxford became our overseeing editor. Such a transition and short timeline for production might rattle the best of editors, but Jason helped to establish the best adaptable plans to keep things on track. Sincere thanks for your efforts, Jason. Meanwhile, in the house of Sinauer, production editors Laura Green and Kathaleen Emerson shared their expertise and their truly collaborative insights, offering us respectful considerations during key times that we will not forget. Thank you Laura for also sharing with us your most valuable plant background throughout the editorial process.

The success of this and each edition equally rests on the quality of the book's design and look, for which we sincerely appreciate the wonderful work Sinauer's art, media, and overall production team have done. The media team was headed by Suzanne Carter and supported by the creative drive of Peter Lacey. Sincere thanks to you both. Further thanks to the entire group at Dragonfly Media, who continue to do a great job taking care to represent many of Michael's original drawings with supreme accuracy. We'd also like to thank Joan Gemme, Beth Roberge, and Annette Rapier for their excellent design, layout, and production of this edition. One of the long-loved hallmarks of *Developmental Biology* has been the incorporation of actual data and images that represent the science. Special thanks to the permissions team, Mark Siddall, Tracy Marton, and Michele Beckta for their non-stop efforts in securing the rights to these essential pieces of the book. But of course, a new book can only reach the hands of the students with the help of a robust and strategic sales team. Many thanks to Susan McGlew and to all the salespersons at Oxford now helping to support this textbook.

Lastly, it needs to be acknowledged that while Scott is blissfully retired, Michael is still working his tail off doing teaching, research, committee assignments, and so forth, in addition to his strong family commitments. He would not be able to provide the time and energy to this textbook if he did not have the support of his own institution and students. Thank you, Smith College, for continuing to allow Michael to produce and disseminate his Web Conferences, Developmental Documentaries, and the Dev Tutorials freely to the community. Most sincere thanks to Michael's research students, who had to endure their principle investigator being too engrossed in all things development all the time! Know that your patience, support, and insights surely made this book possible.

—M.J.F.B.

—S.F.G.

May 24, 2019

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to accompany **Developmental Biology**, *Twelfth Edition*

For the Student

Companion Website

devbio.com

Significantly enhanced for the Twelfth Edition, and referenced throughout the textbook, the *Developmental Biology* Companion Website provides students with a range of engaging resources to help them learn the material presented in the textbook. The companion site is available free of charge and includes resources in the following categories:

- **Dev Tutorials:** Professionally produced video tutorials, presented by the textbook's authors, reinforce key concepts.
- **Watch Development:** Putting concepts into action, these informative videos show real-life developmental biology processes.
- **Further Development:** These extensive topics provide more information for advanced students, historical, philosophical, and ethical perspectives on issues in developmental biology, and links to additional online resources.
- **Scientists Speak:** In these lectures and question-and-answer interviews, developmental biology topics are explored by leading experts in the field.
- **Flashcards:** Per-chapter flashcard sets help students learn and review the many new terms and definitions introduced in the textbook.
- **Literature Cited:** Full citations are provided for all of the literature cited in the textbook (most linked to their PubMed citations).
- **Research Guide:** This illustrated and annotated guide helps students find and comprehend research articles in developmental biology.

For the Instructor

(Available to qualified adopters)

Instructor's Resource Library

The *Developmental Biology*, Twelfth Edition Instructor's Resource Library includes the following resources:

- **Case Studies in Dev Bio:** This collection of case study problems provides instructors with ready-to-use in-class active learning exercises. The case studies foster deep learning in developmental biology by providing students an opportunity to apply course content to the critical analysis of data, to generate hypotheses, and to solve novel problems in the field. Each case study includes a PowerPoint presentation and a student handout with accompanying questions.
- **Developing Questions:** Thought-provoking questions, many with answers, references, and recommendations for further reading, are provided so that you and your students can explore questions that are posed throughout each chapter.
- **Textbook Figures & Tables:** All of the textbook's figures, photos, and tables are provided both in JPEG and PowerPoint formats. All images have been optimized for excellent legibility when projected in the classroom.

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Developmental Biology, Twelfth Edition is also available in a three-hole punched, looseleaf format. Students can take just the sections they need to class and can easily integrate instructor material with the text.

1

The Making of a Body and a Field

Introduction to Developmental Biology

ONE OF THE CRITICAL DIFFERENCES between you and a machine is that a machine is never required to function until after it is built. Every multicellular organism has to function even as it builds itself. It *develops*. In the time between fertilization and birth, the organism is known as an **embryo** (**FIGURE 1.1**). The concept of an embryo is a staggering one. As an embryo, you had to build yourself from a single cell. You had to respire before you had lungs, digest before you had a gut, build bones when you were pulpy, and form orderly arrays of neurons before you knew how to think. It should thus not be surprising that most human embryos die before being born. You survived.

Multicellular organisms do not spring forth fully formed. Rather, they arise by a relatively slow process of progressive change that we call **development**. In most cases, the development of a multicellular organism begins with a single cell—an egg cell that has completed the process of fertilization and is referred to as a **zygote**. The zygote divides mitotically to produce all the cells of the body.

The study of animal development has traditionally been called **embryology**, after that phase of an organism that exists between fertilization and birth. But development does not stop at birth, or even at adulthood. Most organisms never stop developing.

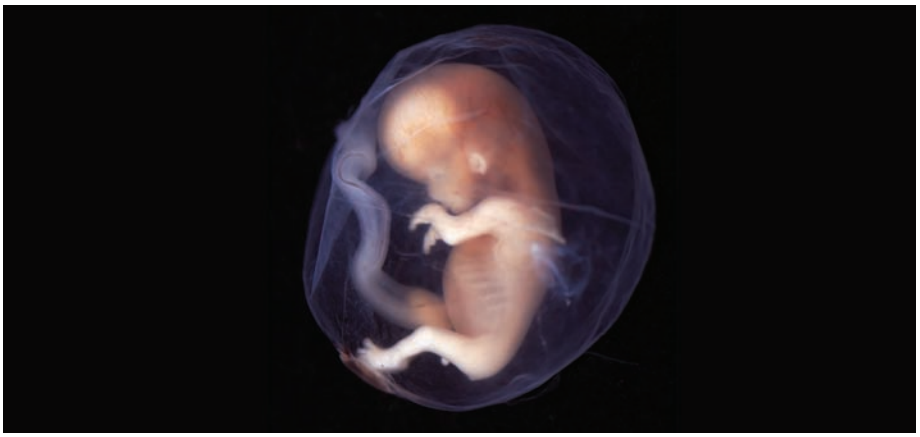
Is this plant really your cousin?

Photo credit: M. J. F. Barresi and Kathryn Lee, 2018.
Thanks to Dr. Robin Sleith for providing the charophyta algae

The Punchline

Development is the route by which an organism goes from genotype to phenotype. In most animals, this involves a fertilized egg that cleaves into many cells. These cells then rearrange during gastrulation and differentiate during organogenesis. Certain animal life cycles may include metamorphic changes and regeneration. In many plants, development also includes fertilization, cleavage, and organogenesis, but within a life cycle that has two alternating stages: a diploid growth stage and a haploid sexual stage. Eventually, most organisms age. Developmental processes are among the greatest sources of questions in science: How are different cell types created, and how are they organized into functional organs? How do organisms make cells that can reproduce or regenerate missing parts? How are environmental cues integrated during development? How can the pathways of development change to produce new types of organisms? And what are the developmental mechanisms for these evolutionary changes? Many of the answers directly relate to our understanding of evolution and human health and disease.

Each day we replace more than a gram of skin cells (the older cells being sloughed off as we move), and our bone marrow sustains the development of millions of new red blood cells every minute of our lives. Plants exhibit an astounding capacity for perpetual growth throughout their life span, a phenomenon known as **indeterminate growth**



lunar caustic/CC BY-SA 2.0

FIGURE 1.1 A 9- to 10-week-old human embryo.

(**FIGURE 1.2A**). Plant cells even have the capacity for whole-organism regeneration (**FIGURE 1.2B**). Some animals can regenerate severed parts (**FIGURE 1.2C**), and many species undergo **metamorphosis** (changing from one form into another, such as the transformation of a tadpole into a frog, or a caterpillar into a butterfly). On the most fundamental level, developmental biology seeks to elucidate the cellular and molecular mechanisms that drive changes in cells, tissues, and organs over time—a timescale that spans all of life, from fertilization through aging.[1]

"How Are You, You?" Comparative Embryology and the Questions of Developmental Biology

Aristotle, the first known embryologist, said that wonder was the source of knowledge, and animal and plant development, as Aristotle knew well, is a remarkable source of wonder. The fertilized egg has no heart. Where does the heart come from? Does it form the same way in both insects and vertebrates? Many of the questions in developmental biology are of this comparative type, and they stem from the field's embryological heritage.

The first known study of comparative developmental anatomy was undertaken by Aristotle. In his book *On the Generation of Animals* (ca. 350 BCE), he noted some of the variations on the life cycle themes: some animals are born from eggs (**oviparity**, as in birds, frogs, and most invertebrates); some by live birth (**viviparity**, as in placental mammals); and some by producing an egg that hatches inside the body (**ovoviviparity**, as in certain reptiles and sharks). Aristotle also identified the two major cell division patterns by which embryos are formed: the **holoblastic** pattern of cleavage (in which the entire egg is divided into successively smaller cells, as it is in frogs and mammals) and the **meroblastic** pattern of cleavage (as in chicks, wherein only part of the egg is destined to become the embryo, while the other portion—the yolk—serves as nutrition for the embryo). And should anyone want to know who first figured out the functions of the mammalian placenta and umbilical cord, it was Aristotle.

There was remarkably little progress in embryology for the two thousand years following Aristotle. It was only in 1651 that William Harvey concluded that all animals—even mammals—originate from eggs. *Ex ovo omnia* (All from the egg) was the motto on the frontispiece of Harvey's *On the Generation of Living Creatures*, and this precluded

[1] Defining exactly what developmental biology encompasses has spurred recent debate (Pradeu et al. 2016). Some feel that the field is impossible to define, while others argue that a framework can help reduce the negative consequences associated with implicit meanings. The authors of this textbook support a more expansive definition, one that promotes inclusion of a diversity of perspectives to better support the collaborative development of the field itself.

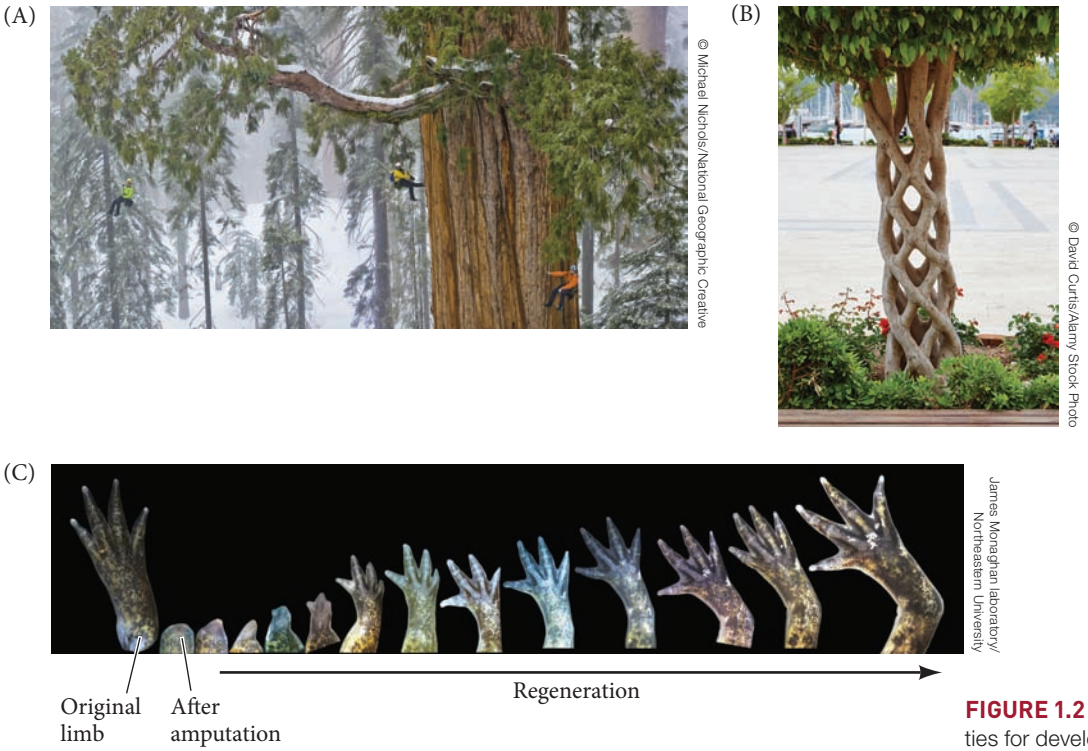


FIGURE 1.2 Extraordinary capacities for development. (A) "Hyperion" has been named the tallest tree in the world. It is a redwood sequoia standing over 114 meters (375 feet) tall, which is 70 feet taller than the Statue of Liberty. The two researchers shown here climbing Hyperion look like spiders hanging from its branches. (B) Axel Erlandson created the "Basket Tree" by cutting the tops off six sycamore trees and forcing engraftment of each tree's regeneration stems together. This demonstrates the remarkable plasticity and regenerative ability of plants. (C) Some animal species also exhibit a remarkable capacity for regeneration. The Mexican salamander can regrow a perfectly constructed limb following its amputation.

the spontaneous generation of animals from mud or excrement.[2] Harvey also was the first to see the blastoderm of the chick embryo (the small region of the egg containing the yolk-free cytoplasm that gives rise to the embryo), and he was the first to notice that "islands" of blood tissue form before the heart does. Harvey also suggested that the amniotic fluid might function as a "shock absorber" for the embryo.

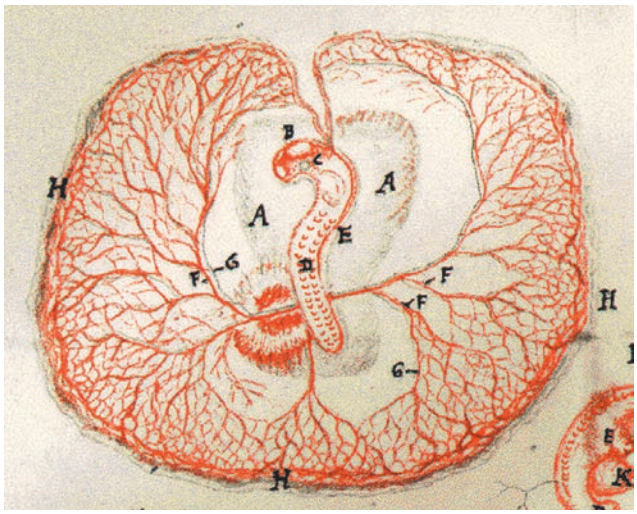
As might be expected, embryology remained little but speculation until the invention of the microscope allowed detailed observations (**FIGURE 1.3**). Marcello Malpighi published the first microscopic account of chick development in 1672. Here, for the first time, the groove of the forming neural tube, the muscle-forming somites, and the first circulation of the arteries and veins—to and from the yolk—were identified.

This development, this formation of an orderly body from relatively homogeneous material, provokes profound and fundamental questions: How does the body form with its head always above its shoulders? Why is the heart on the left side of our body? How does a simple tube become the complex structures of the brain and spinal cord that generate both thought and movement? Why can't we grow back new limbs like a salamander? How do the sexes develop their different anatomies?

Our answers to these questions must respect the complexity of the inquiry and must explain a coherent causal network from gene through functional organ. To say that mammals with two X chromosomes are usually females and those with XY chromosomes are usually males does not explain sex determination to a developmental biologist, who wants to know *how* the XX genotype produces a female and *how* the XY genotype produces a male. Similarly, a geneticist might ask how globin genes are transmitted from one generation to the next, and a physiologist might ask about the function of globin proteins in the body. But the developmental biologist asks how it is that the globin genes come to be expressed only in red blood cells and how these genes become active only at specific times in development. (We don't have all the answers yet.) The particular set of questions asked defines the field of biology, as we, too, become defined (at least in part) by the questions we ask. *Welcome to a wonderful and important set of questions!*

[2]Harvey did not make this statement lightly, for he knew that it contradicted the views of Aristotle, whom Harvey venerated. Aristotle had proposed that menstrual fluid formed the substance of the embryo, while the semen gave it form and animation.

(A)



From M. Malpighi. 1672. *De Formatone Pulli in Ovo*. London

(B)

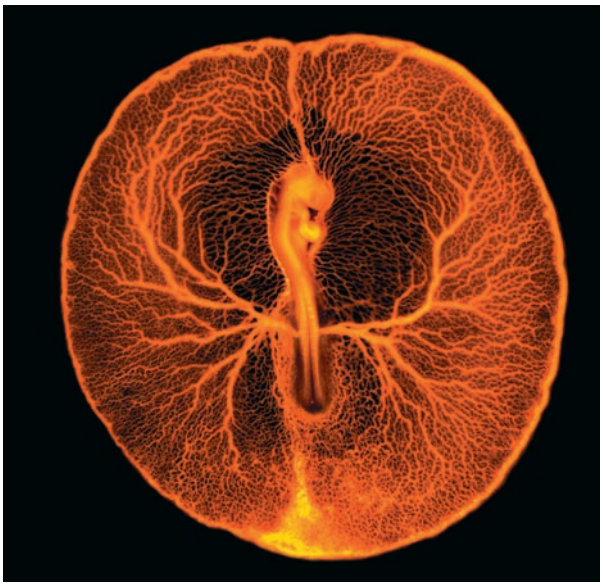
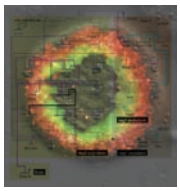


Image courtesy of Vincent Pasque

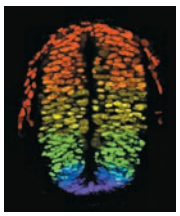
FIGURE 1.3 Depictions of chick developmental anatomy. (A) Dorsal view (looking "down" at what will become the back) of a 2-day chick embryo, as depicted by Marcello Malpighi in 1672. (B) Dorsal view of a late 2-day chick embryo, about 45 hours after the egg was laid. The heart starts beating during day 2. The vascular system of this embryo was revealed by injecting fluorescent beads into the circulatory system. The three-dimensionality is achieved by superimposing two separate images.

Development accomplishes two major objectives. First, it generates cellular diversity and order within the individual organism; second, it ensures the continuity of life from one generation to the next. Put another way, there are two fundamental questions in developmental biology: How does the zygote give rise to the adult body? And how does that adult body produce yet another body? These huge questions can be subdivided into several categories of questions scrutinized by developmental biologists:



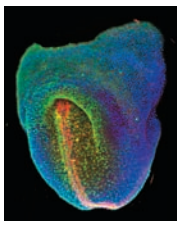
I. S. Peter and E. H. Davidson, 2011. *Nature* 474: 635–639

- **The question of differentiation**. A single cell, the fertilized egg, gives rise to hundreds of different cell types—muscle cells, epidermal cells, neurons, lens cells, lymphocytes, blood cells, fat cells, and so on. This generation of cellular diversity is called **differentiation**. Since every cell of the body (with very few exceptions) contains the same set of genes, how can this identical set of genetic instructions produce different types of cells? How can a single fertilized egg cell generate so many different cell types?[3]



Photograph courtesy of E. M. Gorostiza

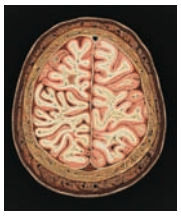
- **The question of pattern formation**. From the stripes that cover a zebra or zebrafish to the anatomical parts of our bodies, cells and tissues are stereotypically positioned in recognizable patterns. Our head is anterior, our tail posterior, and our limbs lateral to the medially positioned nervous system. Our heart is asymmetrically positioned on the left side. Indications of these patterns can be seen early in the embryo. What processes control the elaboration of cell and tissue type patterns?



Photograph courtesy of I. Costello and E. Robertson

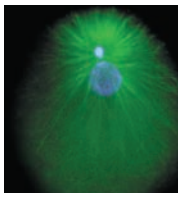
- **The question of morphogenesis**. How can the cells in our body organize into functional structures? Our differentiated cells are not randomly distributed. Rather, they are organized into intricate tissues and organs. During development, cells divide, migrate, and die; tissues fold and separate. The folded tubular shape of our brain and spinal cord started as a flattened plate of cells. Our digestive system functionally connects our mouth and anal openings. This creation of ordered form is called **morphogenesis**, and it involves coordinating cell growth, cell migration, and cell death.

[3] More than 210 different cell types are recognized in the *adult* human, but this number tells us little about how many cell types a human body produces over the course of development.



Photograph courtesy of Lisa Nilsson

- **The question of growth**. If each cell in our face were to undergo just one more cell division, we would be considered horribly malformed. If each cell in our arms underwent just one more round of cell division, we could tie our shoelaces without bending over. How do our cells know when to stop dividing? Our arms are generally the same size on both sides of the body. How is cell division so tightly regulated?

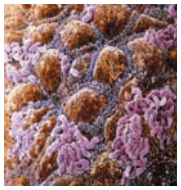


From journal cover associated with J. Holy and G. Schatten. 1991. *Dev Biol* 147 (2), courtesy of J. Holy and G. Schatten

- **The question of reproduction**. The sperm and egg are highly specialized cells that can transmit the instructions for making an organism from one generation to the next. How are these germ cells set apart, and what are the instructions in the nucleus and cytoplasm that allow them to form the next generation?

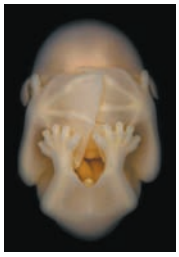
Courtesy of Junji Morokuma and Michael Levin

- **The question of regeneration**. Some organisms can regenerate every part of their bodies. Some salamanders can regenerate their eyes and their legs, and many reptiles can regenerate their tails. While mammals are generally poor at regeneration, there are some cells in our bodies—**stem cells**—that are able to form new structures even in adults. How do stem cells retain this capacity, and can we harness it to cure debilitating diseases?



© P.M. Motta & F. Carpino/Univ. "La Sapienza"/Science Source

- **The question of environmental integration**. The development of many (perhaps all) organisms is influenced by cues from the environment that surrounds the embryo or larva. The sex of many species of turtles, for instance, depends on the temperature the embryo experiences while in the eggshell. The formation of the reproductive system in some insects depends on bacteria that are transmitted inside the egg. Moreover, certain chemicals in the environment can disrupt normal development, causing malformations in the adult. How is the development of an organism integrated into the larger context of its habitat?



Photograph courtesy of R. R. Behringer

- **The question of evolution**. Evolution involves inherited changes of development. When we say that today's one-toed horse had a five-toed ancestor, we are saying that changes in the development of cartilage and muscles occurred over many generations in the embryos of the horse's ancestors. How do changes in development create new body forms? Which heritable changes are possible, given the constraints imposed by the necessity of the organism to survive as it develops?

The questions asked by developmental biologists have become critical in molecular biology, physiology, cell biology, genetics, anatomy, cancer research, neurobiology, immunology, ecology, and evolutionary biology. Each of these disciplines has its ancestral roots in developmental biology. Yet unlike each of these descendant disciplines, which seem to continually differentiate into further sets of restricted paradigms, developmental biology remains pluripotent. In fact, it has recently been proposed that developmental biology is the "stem cell of biological disciplines" (Gilbert 2017).

CHOOSING THE ORGANISM TO STUDY THE QUESTION: THE "MODEL" SYSTEM

To answer the questions that developmental biologists ask, researchers need a tractable experimental organism best suited to their questions. What makes an organism a good "model" for addressing a given question? Just as an axe and a chain saw are suited to similar but different tasks, different animal model systems provide investigators with different advantages. Some of the common considerations in choosing a good model system are the following:

Size: A particularly practical consideration is the size of the adult organism. Is it easy to house a significant number of breeding adults in the allotted laboratory infrastructure? For example, housing 50 mice in cages requires a lot more space and expense then housing 50 flies in a vial.

Generation time: How long does it take the organism to complete its life cycle from embryo to reproductive adult? Additionally, how short is the embryonic period? The roundworm *Caenorhabditis elegans* has a full life cycle of 3 days, whereas it takes the zebrafish about 3 months to go from "egg to egg." However, early embryogenesis in a zebrafish spans only 24 hours.

Embryo accessibility: To study embryology, a researcher needs to be able to see and work with the actual embryo. Different species pose different challenges for embryo accessibility. Some embryos are dispersed in the water for easy collection, while others develop in an opaque shell, such as the avian egg, or in utero (within the womb or uterus), as with mammals.

Feasibility of genomic interrogation: Since Mendel's work with peas, developmental biologists have been driven to identify the genetic basis underlying all developmental processes, from embryology to disease. Although all life is based on the organization and use of the four nucleotide bases, no species has the same genome. Genome size, organization, and content all differ, which can affect the level of genetic interrogation that is possible. For instance, researchers studying regeneration in the Mexican salamander have to deal with the largest genome ever sequenced. Maybe the secret of regeneration lies somewhere in all that DNA.

Organism type and phylogenetic position: Ideally, the research question should guide the selection of a model system. If researchers are interested in the remarkable process of metamorphosis, then clearly they are limited to a select few model species that display such transformations, such as the fruitfly or frog. If they are passionate about studying human development, they may use a mammalian model organism, such as the mouse, or human cells in culture. If their questions are focused on deciphering the developmental changes fueling evolution, they can choose species that occupy informative phylogenetic positions, such as the charophytic algae that are basal to multicellular land plants.

Ease of experimental manipulation: Last, but certainly not least, among the considerations is whether an organism is appropriate for the experimental approach needed to answer the question being asked. For example, due to the long history of significant investments to develop the fruitfly and mouse model systems, a plethora of powerful molecular and genetic tools exist to manipulate gene and protein function during embryonic development of these organisms. Similarly, the extensive body of information now available on the genetics and development of the small mustard plant *Arabidopsis thaliana* has made it a widely used model organism in research on flowering plants.

THE USUAL SUSPECTS Some of the more common model systems used to study embryonic development include a flowering weed (*Arabidopsis thaliana*), sea urchin (*Strongylocentrotus purpuratus*), sea squirt (*Ciona intestinalis*), fruitfly (*Drosophila melanogaster*), roundworm (*Caenorhabditis elegans*), zebrafish (*Danio rerio*), African clawed frog (*Xenopus laevis*), chicken (*Gallus gallus*), and mouse (*Mus musculus*) (**FIGURE 1.4**). This short list of usual suspects is not a true representation of the diversity of organisms actually being used to study developmental biology, however. For instance, hydra, planarian flatworms, the *Axolotl* salamander, and the spiny mouse are among the top animals used to study regeneration. Many of the above model systems are actively being used to directly model the development of human disease. Additionally, human pluripotent stem cells are being used to study human development in a dish.

Advances in shared genomic and molecular approaches have dramatically increased the accessibility of nontraditional or nonmodel organisms for developmental research. This is one of the most exciting things about being a new student entering the field of developmental biology today. You do not have to be restricted to the conventional model systems; rather, any species could be a new model organism for you to investigate.

FIGURE 1.4 Some of the model systems used to study developmental biology. From left to right the silhouettes represent the following model organisms: *Arabidopsis thaliana*, *Drosophila melanogaster*, *Hydra vulgaris*, *Caenorhabditis elegans*, *Xenopus laevis*, *Danio rerio*, *Gallus gallus*, *Mus musculus*, and stem cells of *Homo sapiens* (blastocyst with inner cell mass depicted).

The Cycle of Life

Through initial studies of model organisms, descriptive embryology has brought us an understanding of the life cycles of various organisms.

An animal's life cycle

Most animals, whether earthworm or eagle, termite or beagle, pass through similar stages of development: fertilization, cleavage, gastrulation, organogenesis, hatching (or birth), metamorphosis, and gametogenesis. The stages of development between fertilization and hatching (or birth) are collectively called **embryogenesis**.

1. **Fertilization** involves the fusion of the mature sex cells, the sperm and egg, which are collectively called the **gametes**. The fusion of the gamete cells stimulates the egg to begin development and initiates a new individual. The subsequent fusion of the gamete nuclei (the male and female **pronuclei**, each of which has only half the normal number of chromosomes characteristic for the species) gives the embryo its **genome**, the collection of genes that helps instruct the embryo to develop in a manner very similar to that of its parents.
2. **Cleavage** is a series of mitotic divisions that immediately follow fertilization. During cleavage, the enormous volume of zygote cytoplasm is divided into numerous smaller cells called **blastomeres**. By the end of cleavage, the blastomeres have usually formed a sphere, known as a **blastula**.[4]
3. After the rate of mitotic division slows down, the blastomeres undergo dramatic movements and change their positions relative to one another. This series of extensive cell rearrangements is called **gastrulation**, and the embryo is said to be in the **gastrula** stage. As a result of gastrulation, the embryo contains three **germ layers** (**endoderm**, **ectoderm**, and **mesoderm**) that will interact to generate the organs of the body.
4. Once the germ layers are established, the cells interact with one another and rearrange themselves to produce tissues and organs. This process is called **organogenesis**. Chemical signals are exchanged between the cells of the germ layers, resulting in the formation of specific organs at specific sites. Certain cells will undergo long migrations from their place of origin to their final location. These migrating cells include the precursors of blood cells, lymph cells, pigment cells, and gametes (eggs and sperm).
5. In most species, the organism that hatches from the egg or is born into the world is not sexually mature. Rather, the organism needs to undergo metamorphosis to become a sexually mature adult. In most animals, the young organism is called a **larva**, and it may look significantly different from the adult. In some species, the larval stage is the one that lasts the longest, and is used for feeding or dispersal. In such species, the adult is a brief stage whose sole purpose is to reproduce. In silkworm moths, for instance, the adults do not have mouthparts and cannot feed; the

[4] We will be using an entire "blast" vocabulary in this book. A *blastomere* is a cell derived from cleavage in an early embryo. A *blastula* is an embryonic stage composed of blastomeres; a mammalian blastula is called a *blastocyst* (see Chapter 12). The cavity within the blastula is the *blastocoel*. A blastula that lacks a blastocoel is called a *stereoblastula*. The invagination where gastrulation begins is the *blastopore*.

larva must eat enough so that the adult has the stored energy to survive and mate. Indeed, most female moths mate as soon as they eclose from the pupa, and they fly only once—to mate and lay their eggs. Then they die.

6. In many species, a group of cells is set aside to produce the next generation (rather than forming the current embryo). These cells are the precursors of the gametes. The gametes and their precursor cells are collectively called **germ cells**, and they are set aside for reproductive function. All other cells of the body are called **somatic cells**. This separation of somatic cells (which give rise to the individual body) and germ cells (which contribute to the formation of a new generation) is often one of the first differentiations to occur during animal development. The germ cells eventually migrate to the gonads, where they differentiate into gametes. The development of gametes, called **gametogenesis**, is usually not completed until the organism has become physically mature. At maturity, the gametes may be released and participate in fertilization to begin a new embryo. The adult organism eventually undergoes senescence and dies, its nutrients often supporting the early embryogenesis of its offspring and its absence allowing less competition. Thus, the cycle of life is renewed. (See **Further Development 1.1, When Does a Human Become a Person?**, online.)

DEV TUTORIAL **Personhood** Scott Gilbert discusses the human life cycle and the question of when in this cycle the embryo may be said to achieve "personhood."

A flowering plant's life cycle

The life cycle of flowering plants (and of all other land plants) is different from that of animals in having two alternating stages, a diploid **sporophytic** (diploid spore-bearing) stage and a haploid **gametophytic** (haploid gamete-producing) stage. When you picture a beautiful rose with its flower, leaves, stem, and hidden roots, you are looking at the full-grown sporophytic stage; within its flowers are the female and male gametophytes that produce eggs and sperm. Upon fertilization, these gametes create the embryos of the next generation of sporophytes, held within the seed coats that protect them (see Figure 1.8). Under optimal environmental conditions these embryos develop, and a new cycle of life can commence.

The life cycle of a flowering plant is similar in various aspects to the general scheme of an animal's life cycle. Both male and female haploid gametes are produced, the male gamete must travel to the female gamete, and subsequent fertilization initiates mitotic cell divisions and the development of the embryo. As in animals, the embryo develops three basic cell layers, but these do not rearrange through gastrulation-like movements. In addition, the embryo, which is developing within a seed, typically pauses between completion of embryogenesis and subsequent germination and growth. This dormancy period can be exceedingly long. Unlike animals, plants have indeterminate growth. This continued growth is possible because plants retain areas of stem cells for growth called meristems, which are located at the apical and basal tips of the embryo and are maintained in the adult. (Although adult animals also retain stem cells, these are not used for indeterminate growth.) Differentiation of tissues in the developing plant results in organogenesis like in animals, but plant cells have a cell wall outside their plasma membrane that is nonexistent in animals. This plant cell wall imposes many constraints on the developmental mechanisms driving plant patterning and growth, such as inhibiting cell movement, restricting planes of cell division, requiring unique modes of molecule transport between cells, and more robust responses for regenerative repair, to name just a few.

Example 1: A Frog's Life

All animal life cycles are modifications of the generalized one described above. Here we present a concrete example, the development of the leopard frog *Rana pipiens* (**FIGURE 1.5**).

WATCH DEVELOPMENT 1.1 Watch an entire salamander develop from a single cell in six minutes.

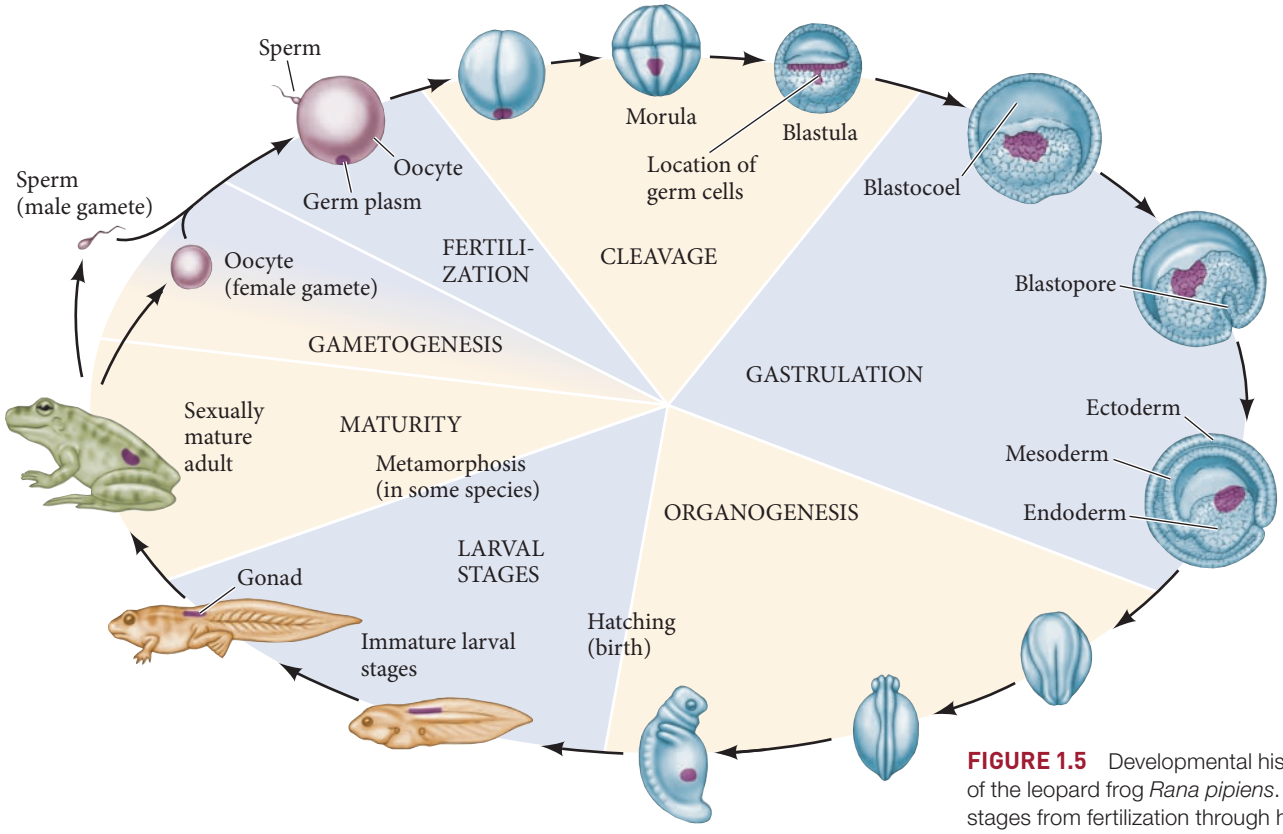


FIGURE 1.5 Developmental history of the leopard frog *Rana pipiens*. The stages from fertilization through hatching (birth) are known collectively as embryogenesis. The region set aside for producing germ cells is shown in purple. Gametogenesis, which is completed in the sexually mature adult, begins at different times during development, depending on the species. (The sizes of the varicolored wedges shown here are arbitrary and do not correspond to the proportion of the life cycle spent in each stage.)

Gametogenesis and fertilization

The end of one life cycle and the beginning of the next are often intricately intertwined. Life cycles are often controlled by environmental factors (tadpoles wouldn't survive if they hatched in the fall, when their food is dying), so in most frogs, gametogenesis and fertilization are seasonal events. A combination of photoperiod (hours of daylight) and temperature informs the pituitary gland of the mature female frog that it is spring. The pituitary secretions cause the eggs and sperm to mature.

In most species of frogs, fertilization is external (**FIGURE 1.6A**). The male frog grabs the female's back and fertilizes the eggs as the female releases them (**FIGURE 1.6B**). Some species lay their eggs in pond vegetation, and the egg jelly adheres to the plants and anchors the eggs. The eggs of other species float into the center of the pond without any support. So an important thing to remember about life cycles is that they are intimately intertwined with environmental factors.

Fertilization accomplishes both sex (genetic recombination) and reproduction (the generation of a new individual). The genomes of the haploid male and female pronuclei merge and recombine to form the diploid zygote nucleus. In addition, the entry of the sperm facilitates the movement of cytoplasm inside the newly fertilized egg. This migration will be critical in determining the three body axes of the frog: anterior-posterior (head-tail), dorsal-ventral (back-belly), and right-left. And importantly, fertilization activates those molecules necessary to begin cell cleavage and gastrulation (Rugh 1950).

Cleavage and gastrulation

During cleavage, the volume of the frog egg stays the same, but it is divided into tens of thousands of cells (**FIGURE 1.6C,D**). Gastrulation in the frog begins at a point on the embryo surface roughly 180° opposite the point of sperm entry with the formation of a dimple called the **blastopore** (**FIGURE 1.6E**). The blastopore, which marks the future dorsal side of the embryo, expands to become a ring. Cells migrating through the blastopore to the embryo's interior become the mesoderm and endoderm; cells remaining outside become the ectoderm, and this outer layer expands to enclose the entire embryo. Thus, at the end of gastrulation, the ectoderm (precursor of the epidermis, brain, and nerves) is on the outside of the embryo, the endoderm (precursor of the lining of the gut

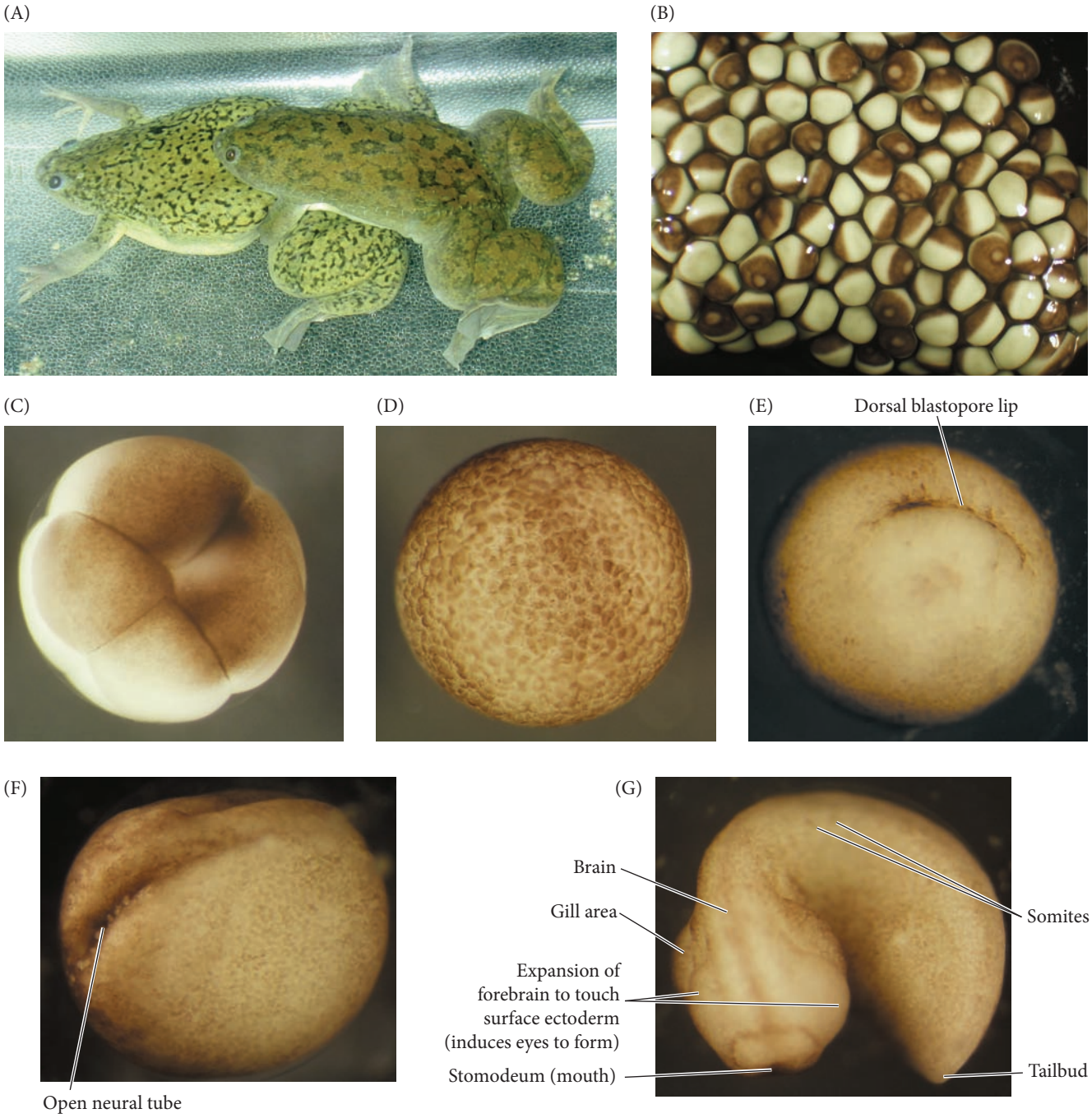


FIGURE 1.6 Early development of the frog *Xenopus laevis*. (A) Frogs mate by amplexus, the male grasping the female around the belly and fertilizing the eggs as they are released. (B) A newly laid clutch of eggs. The cytoplasm has rotated such that the darker pigment is where the nucleus resides. (C) An 8-cell embryo. (D) A late blastula, containing thousands of cells. (E) An early gastrula, showing the blastopore lip through which the mesodermal and some endoderm cells migrate. (F) A neurula, where the neural folds come together at the dorsal midline, creating a neural tube. (G) A pre-hatching tadpole, as the protrusions of the forebrain begin to induce eyes to form. (H) A mature tadpole, having swum away from the egg mass and feeding independently.

All photos courtesy of Michael Danilchik and Kimberly Ray (*Xenopus* egg development)

and respiratory systems) is deep inside the embryo, and the mesoderm (precursor of the connective tissue, muscle, blood, heart, skeleton, gonads, and kidneys) is between them.

Organogenesis

Organogenesis in the frog begins when the cells of the most dorsal region of the mesoderm condense to form the rod of cells called the notochord.[5] These notochord cells produce chemical signals that redirect the fate of the ectodermal cells above it. Instead of forming epidermis, the cells above the notochord are instructed to become the cells of the nervous system. The cells change their shapes and rise up from the round body (**FIGURE 1.6F**). At this stage, the embryo is called a **neurula**. The neural precursor cells elongate, stretch, and fold into the embryo, forming the **neural tube**. The future epidermal cells of the back cover the neural tube.

Once the neural tube has formed, it and the notochord induce changes in the neighboring regions, and organogenesis continues. The mesodermal tissue adjacent to the neural tube and notochord becomes segmented into **somites**—the precursors of the frog's back muscles, spinal vertebrae, and dermis (the inner portion of the skin). The embryo develops a mouth and an anus, and it elongates into the familiar tadpole structure (**FIGURE 1.6G**). The neurons make connections to the muscles and to other neurons, the gills form, and the larva is ready to hatch from its egg. The hatched tadpole will feed for itself as soon as the yolk supplied by its mother is exhausted.

Metamorphosis and gametogenesis

Metamorphosis of the fully aquatic tadpole larva into an adult frog that can live on land is one of the most striking transformations in all of biology. Almost every organ is subject to modification, and the resulting changes in form are striking (**FIGURE 1.7**). The hindlimbs and forelimbs the adult will use for locomotion differentiate as the tadpole's paddle tail recedes. The cartilaginous tadpole skull is replaced by the predominantly bony skull of the young frog. The horny teeth the tadpole uses to tear up pond plants

[5] Although adult vertebrates do not have a notochord, this embryonic organ is critical for establishing the fates of the ectodermal cells above it, as we will see in Chapter 13.

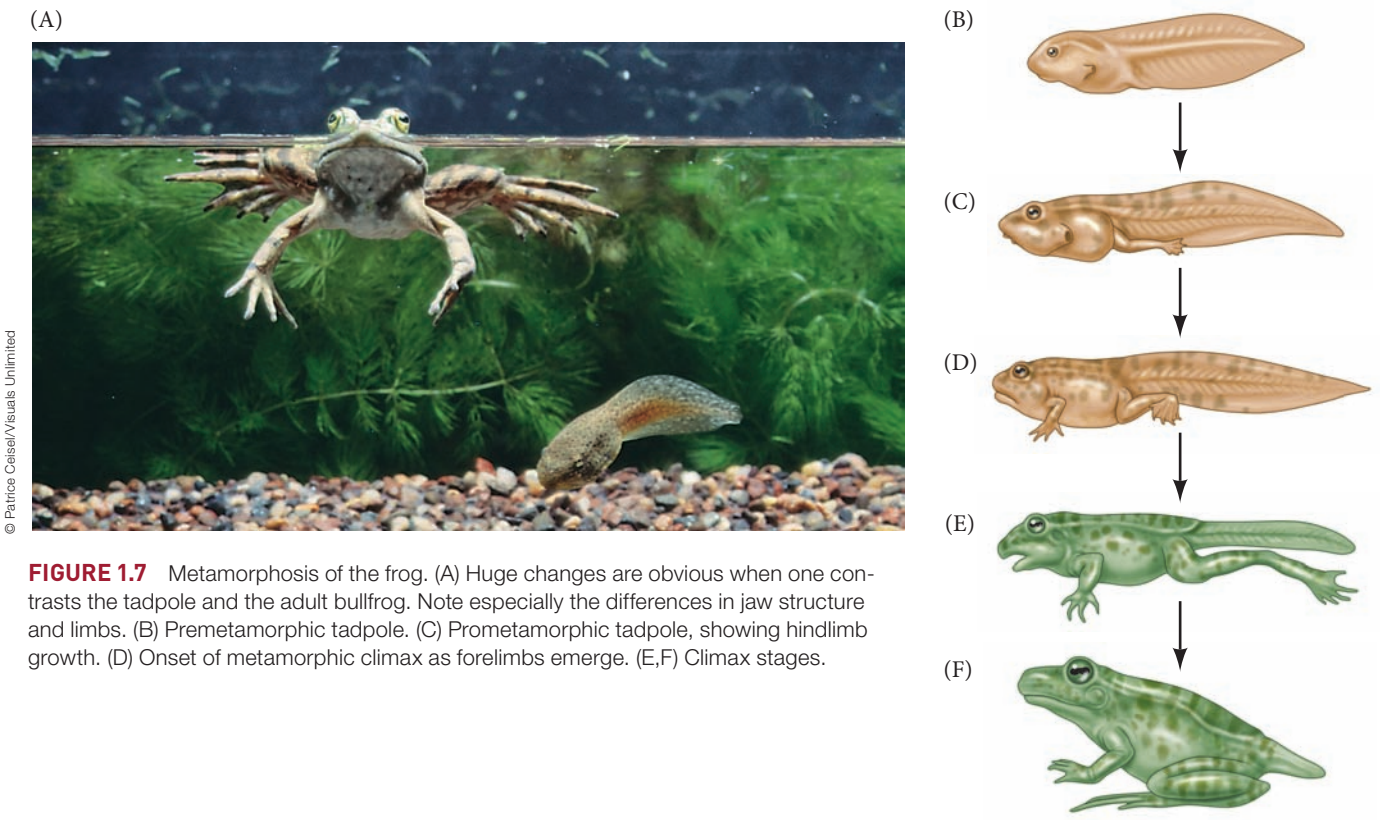


FIGURE 1.7 Metamorphosis of the frog. (A) Huge changes are obvious when one contrasts the tadpole and the adult bullfrog. Note especially the differences in jaw structure and limbs. (B) Premetamorphic tadpole. (C) Prometamorphic tadpole, showing hindlimb growth. (D) Onset of metamorphic climax as forelimbs emerge. (E,F) Climax stages.

disappear as the mouth and jaw take a new shape, and the fly-catching tongue muscle of the frog develops. Meanwhile, the tadpole's lengthy intestine—a characteristic of herbivores—shortens to suit the more carnivorous diet of the adult frog. The gills regress and the lungs enlarge. Amphibian metamorphosis is initiated by hormones from the tadpole's thyroid gland; the mechanisms by which thyroid hormones accomplish these changes will be discussed in Chapter 21. The speed of metamorphosis is keyed to environmental pressures. In temperate regions, for instance, *Rana* metamorphosis must occur before ponds freeze in winter. An adult leopard frog can burrow into the mud and survive the winter; its tadpole cannot.

As metamorphosis ends, the development of the germ cells (sperm and eggs) begins. Gametogenesis can take a long time. In *Rana pipiens*, it takes 3 years for the eggs to mature in the female's ovaries. Sperm take less time; *Rana* males are often fertile soon after metamorphosis. To become mature, the germ cells must be competent to complete **meiosis**, the cell divisions that halve the number of chromosomes to produce haploid gametes. Having undergone meiosis, the mature sperm and egg nuclei can unite in fertilization, restoring the diploid chromosome number and initiating the events that lead to development and the continuation of the circle of life.

Example 2: Even a Weed Can Have a Flower-Full Life

Much of our discussions of plant development in this text will focus on research conducted on the angiosperm *Arabidopsis thaliana*. This small flowering plant, considered a weed, has all the criteria for a great laboratory model organism. Its life cycle is only 6 weeks long, its techniques for propagation are routine, and it has a comparatively small genome that has been sequenced and annotated many times over. The diversity of genetic, environmental, and other experimental approaches available to *A. thaliana* researchers has provided a wealth of understanding behind the mechanisms driving all aspects of this vascular plant's life cycle. Importantly, due to the monophyletic (descended from a single common ancestor) relationships of land plants, much of what has been learned about *A. thaliana* development is relevant to all plants (Koornneef and Meinke 2010; Provart et al. 2016). However, a flowering weed is not a sycamore tree, nor is it corn; there is diversity in the mechanisms of embryogenesis among different plants, and we will be highlighting some of these in later chapters.

Reproductive and gametophytic phases

When an adult flowering plant (angiosperm) is in the reproductive phase, the plant will have fully developed flowers with pollen-producing stamens (male reproductive organs) and ovary-containing carpels (female reproductive organs), which produce the haploid sperm and egg, respectively (**FIGURE 1.8**). These gametes are produced in the gametophytic phase. When pollen carrying the sperm delivers sperm to an egg, fertilization occurs, yielding a diploid zygote (single-celled embryo) (see Chapter 7; Huijser and Schmid 2011).

Embryogenesis and seed maturation

In contrast to cleavage in some animals that have large amounts of yolk in their eggs, which impedes cleavage, embryonic cleavage in seed-producing plants is not constrained by yolk, as the nutrient supply to the plant embryo comes from the surrounding endosperm in the seed (see Figure 1.8; Palovaara et al. 2016). However, of critical significance is the fact that the zygote divides, but does so asymmetrically. The first cell division yields a small (approximately one-third-size) apical cell and a much larger basal cell. The apical cell goes on to generate the embryo proper, while the basal cell becomes the suspensor, which functions to support the embryo, in part by ensuring that it develops within the lumen of the seed. This initial asymmetrical division sets up the primary apical-basal axis of the embryo, such that shoots (stem, leaves, and flowers) will grow from the apical-most cells, while roots will develop from the basal-most cells. Precisely positioned transverse and longitudinal division planes continue to build the embryo through the globular, heart, torpedo, and mature stages (see Figure 1.8). Since plant cells cannot migrate or move, there are no gastrulation movements as you would find in animal embryogenesis; instead, the different morphologies at

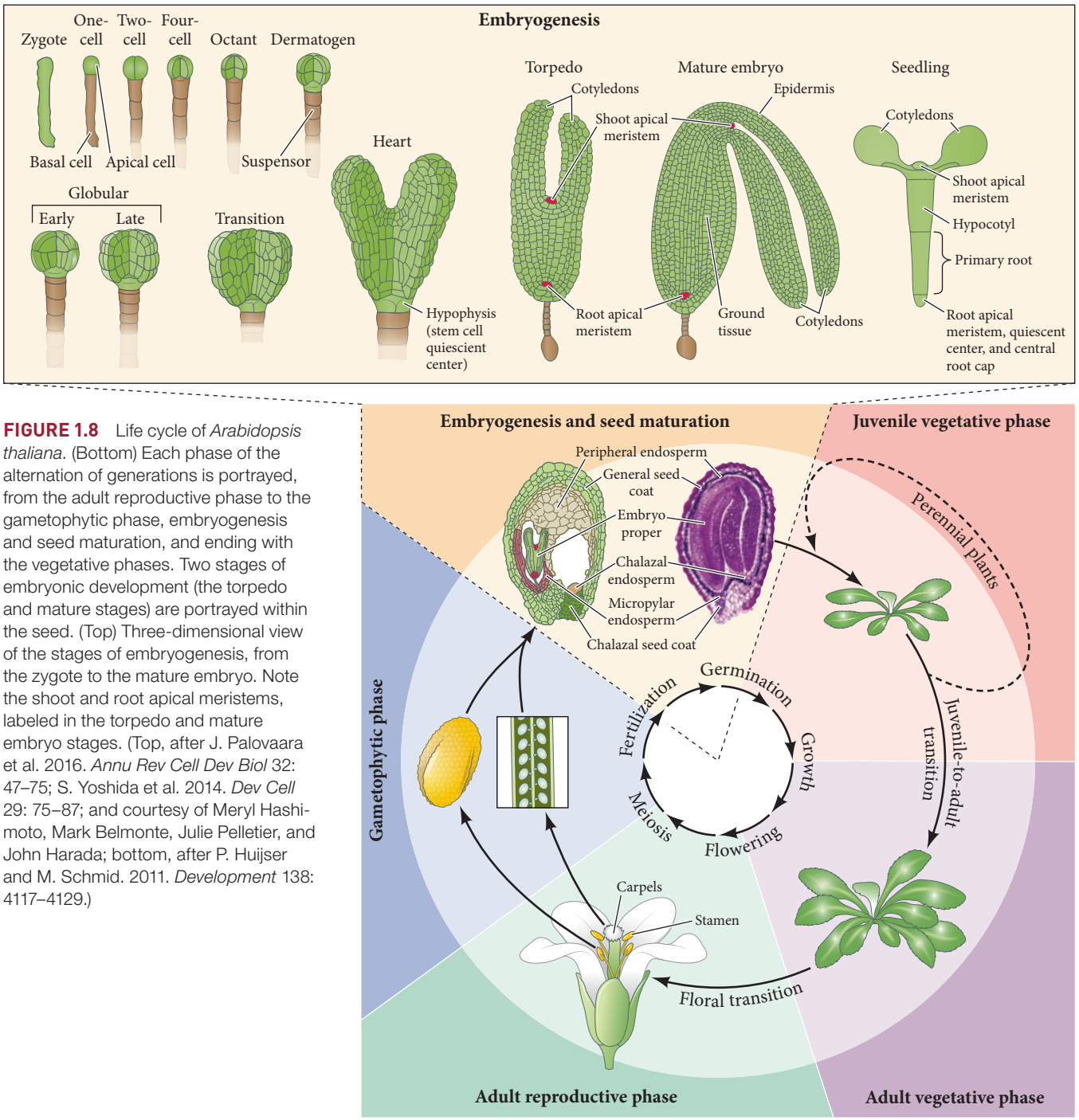


FIGURE 1.8 Life cycle of *Arabidopsis thaliana*. (Bottom) Each phase of the alternation of generations is portrayed, from the adult reproductive phase to the gametophytic phase, embryogenesis and seed maturation, and ending with the vegetative phases. Two stages of embryonic development (the torpedo and mature stages) are portrayed within the seed. (Top) Three-dimensional view of the stages of embryogenesis, from the zygote to the mature embryo. Note the shoot and root apical meristems, labeled in the torpedo and mature embryo stages. (Top, after J. Palovaara et al. 2016. *Annu Rev Cell Dev Biol* 32: 47–75; S. Yoshida et al. 2014. *Dev Cell* 29: 75–87; and courtesy of Meryl Hashimoto, Mark Belmonte, Julie Pelletier, and John Harada; bottom, after P. Huijser and M. Schmid. 2011. *Development* 138: 4117–4129.)

these stages are all based on manipulation of the plane of division as well as on the directionality of cell growth.

Among the major structures that form during *A. thaliana* embryogenesis are the stem and root meristematic tissues and the embryonic leaves called **cotyledons** (see Figure 1.8). The basal-most cluster of cells of the embryo takes on stem cell behaviors and is called the **root apical meristem** (**RAM**); the cells positioned along the central axis of the embryo at the apical-most region are called the **shoot apical meristem** (**SAM**) and similarly possess self-renewing and differentiation behaviors (see Figure 1.8). Additionally, the lateral apexes producing the heart-shaped morphology give rise to the two cotyledons, which provide nutrients to support development through embryogenesis and germination of the seedling (see Figure 1.8).

Plants do not have a huge variety of different cell types, but three distinctive tissue types immediately become segregated in the embryo: **dermal**, **ground**, and **vascular tissues**. The dermal cells will produce the outer layers of the plant epidermis. Ground tissues give rise to the bulk of a plant's internal structures. The cells at the very core of the embryo will form the vascular tissues: **xylem**, which are conduits for bringing water and nutrients upward through the plant, and **phloem**, which are conduits for bringing sugars produced by photosynthesis and other metabolites, primarily from the leaves to parts of the plant that consume more than they produce.

Vegetative phases: From sporophytic growth to inflorescence identity

Upon completion of germination, the now-established sporophyte grows. This marks the beginning of the juvenile vegetative phase, which generally increases the plant's mass and overall size as it continues into the adult vegetative phase. The next phase is the adult reproductive phase, during which a change in the differentiation program of the SAM cells occurs, so that they start generating reproductive tissues instead of stems and leaves. This means the plant starts producing flowers, with their gamete-producing stamens and carpels. Once the plant is fully developed, the life cycle can repeat.

An Overview of Early Animal Development

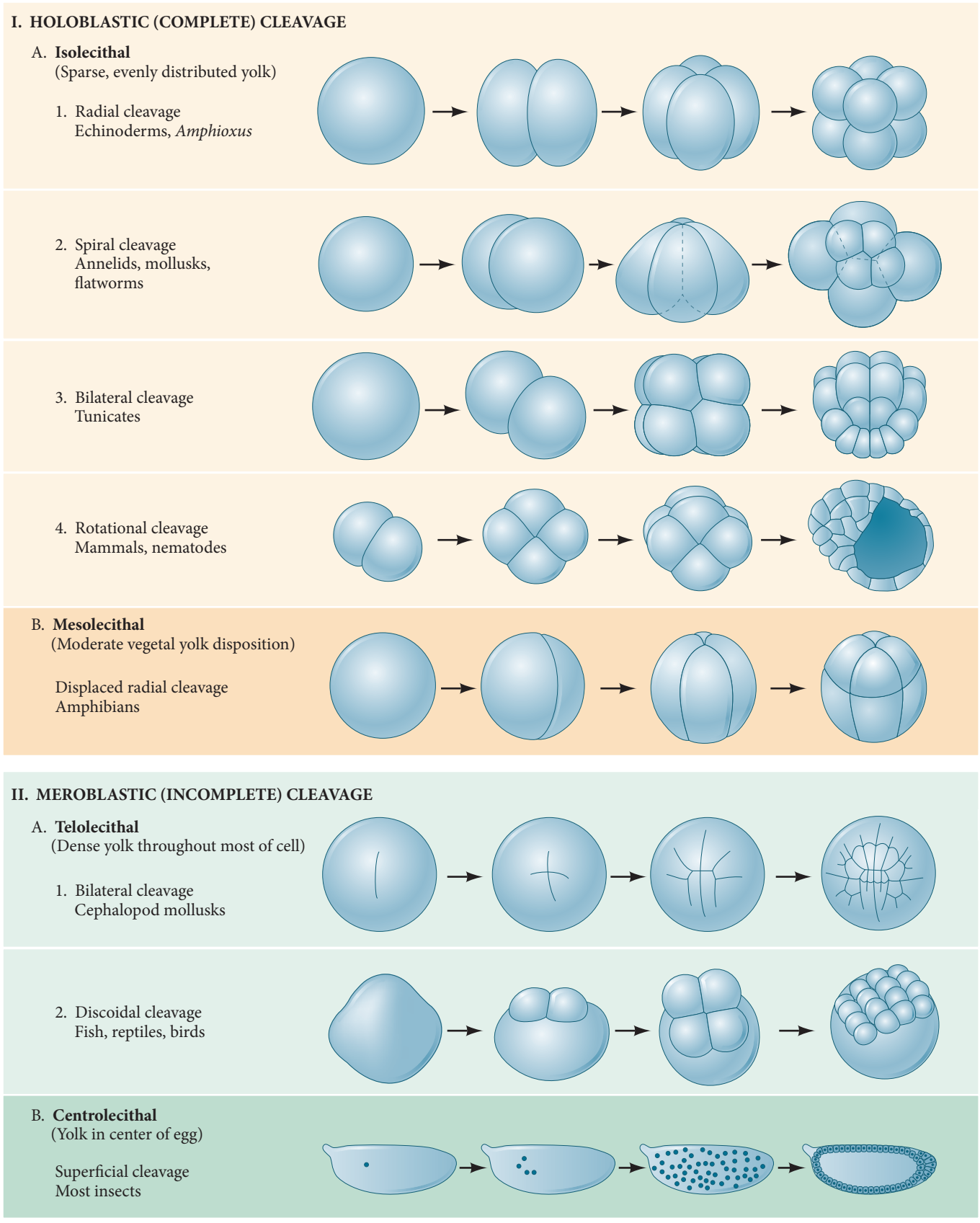
Patterns of cleavage

E. B. Wilson, one of the pioneers in applying cell biology to embryology, noted in 1923, "To our limited intelligence, it would seem a simple task to divide a nucleus into equal parts. The cell, manifestly, entertains a very different opinion." Indeed, different organisms undergo cleavage in distinctly different ways, and the mechanisms for these differences remain at the frontier of cell and developmental biology. Cells in the cleavage stage are called blastomeres. In most animal species (mammals being the chief exception), both the initial rate of cell division and the placement of the blastomeres with respect to one another are under the control of proteins and mRNAs stored in the oocyte. Only later do the rates of cell division and the placement of cells come under the control of the newly formed organism's own genome (that is, the zygotic genome). During the initial phase of development, when cleavage rhythms are controlled by maternal factors, cytoplasmic volume does not increase. Rather, the zygote cytoplasm is divided into ever smaller cells—first in halves, then quarters, then eighths, and so forth. Cleavage occurs very rapidly in most invertebrates and many vertebrates, probably as an adaptation to generate a large number of cells quickly and to restore the somatic ratio of nuclear volume to cytoplasmic volume. The embryo often accomplishes this by abolishing the gap periods of the cell cycle (the G1 and G2 phases), when growth can occur. A frog egg, for example, can divide into 37,000 cells in just 43 hours. Mitosis in cleavage-stage *Drosophila* embryos occurs every 10 minutes for more than 2 hours, forming some 50,000 cells in just 12 hours.

The pattern of embryonic cleavage peculiar to a species is determined by two major parameters: (1) the amount and distribution of yolk protein within the cytoplasm, which determine where cleavage can occur and the relative sizes of the blastomeres; and (2) factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation.

In general, yolk impedes cleavage. When one pole of the egg is relatively yolk-free, cellular divisions occur there at a faster rate than at the opposite pole. The yolk-rich pole is referred to as the **vegetal pole**; the yolk concentration in the **animal pole** is relatively low. The zygote nucleus is frequently displaced toward the animal pole. **FIGURE 1.9** provides a classification of cleavage types and shows the influence of yolk on cleavage symmetry and pattern. At one extreme are the eggs of some sea

FIGURE 1.9 Summary of the main patterns of cleavage.

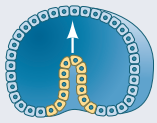
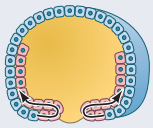
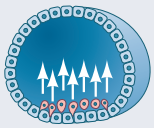
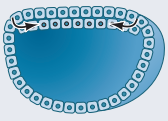
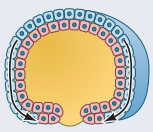


urchins, mammals, and snails. These eggs have sparse, equally distributed yolk and are thus **isolecithal** (Greek, "equal yolk"). In these species, cleavage is holoblastic (Greek *holos*, "complete"), meaning that the cleavage furrow extends through the entire egg. With little yolk, these embryos must have some other way of obtaining food. Most will generate a voracious larval form, while placental mammals will obtain their nutrition from the maternal placenta.

At the other extreme are the eggs of insects, fish, reptiles, birds, and egg-laying mammals (monotremes). Most of their cell volumes are made up of yolk. The yolk must be sufficient to nourish these animals throughout embryonic development. Zygotes containing large accumulations of yolk undergo meroblastic cleavage (Greek *meros*, "part"), wherein only a portion of the cytoplasm is cleaved. The cleavage furrow does not penetrate the yolky portion of the cytoplasm because the yolk platelets impede membrane formation there. Insect eggs have yolk in the center (i.e., they are **centrolecithal**), and the divisions of the cytoplasm occur only in the rim of cytoplasm, around the periphery of the cell (i.e., **superficial cleavage**). The eggs of birds and fish have only one small area of the egg that is free of yolk (**telolecithal** eggs), and therefore the cell divisions occur only in this small disc of cytoplasm, giving rise to **discoidal cleavage**. These are general rules, however, and even closely related species have evolved different patterns of cleavage in different environments.

Yolk is just one factor influencing a species' pattern of cleavage. There are also inherited patterns of cell division superimposed on the constraints of the yolk. The importance of this inheritance can readily be seen in isolecithal eggs. In the absence of a large concentration of yolk, holoblastic cleavage takes place. Four major patterns

TABLE 1.1 Types of cell movement during gastrulation[a]

Type of movement	Description	Illustration	Example
Invagination	Infolding of a sheet (epithelium) of cells, much like the indention of a soft rubber ball when it is poked.		Sea urchin endoderm
Involution	Inward movement of an expanding outer layer so that it spreads over the internal surface of the remaining external cells.		Amphibian mesoderm
Ingression	Migration of individual cells from the surface into the embryo's interior. Individual cells become mesenchymal (i.e., separate from one another) and migrate independently.		Sea urchin mesoderm, *Drosophila* neuroblasts
Delamination	Splitting of one cellular sheet into two more or less parallel sheets. While on a cellular basis it resembles ingression, the result is the formation of a new (additional) epithelial sheet of cells.		Hypoblast formation in birds and mammals
Epiboly	Movement of epithelial sheets (usually ectodermal cells), spreading as a unit (rather than individually) to enclose deeper layers of the embryo. Can occur by cells dividing, by cells changing their shape, or by several layers of cells intercalating into fewer layers; often, all three mechanisms are used.		Ectoderm formation in sea urchins, tunicates, and amphibians

[a]The gastrulation of any particular organism is an ensemble of several of these movements.

of this cleavage type can be described: *radial*, *spiral*, *bilateral*, and *rotational* holoblastic cleavage (see Figure 1.9). (See **Further Development 1.2, The Cell Biology of Embryonic Cleavage**, online.)

Gastrulation: "The most important time in your life"

According to embryologist Lewis Wolpert, "It is not birth, marriage, or death, but gastrulation which is truly the most important time in your life." This is not an overstatement. Gastrulation is what makes animals animals. (Animals gastrulate; plants and fungi do not.) During gastrulation, the cells of the blastula are given new positions and new neighbors, and the multilayered body plan of the organism is established. The cells that will form the endodermal and mesodermal organs are brought to the inside of the embryo, while the cells that will form the epidermis (outer layer of skin) and nervous system are spread over its outside surface. Thus, the three germ layers—outer ectoderm, inner endoderm, and, in between them, mesoderm—are first produced during gastrulation. In addition, the stage is set for the interactions of these newly positioned tissues.

Gastrulation usually proceeds by some combination of several types of movements. These movements involve the entire embryo, and cell migrations in one part of the gastrulating embryo must be intimately coordinated with other movements that are taking place simultaneously. Although patterns of gastrulation vary enormously throughout the animal kingdom, all of the patterns are different combinations of the five basic types of cell movements—**invagination**, **involution**, **ingression**, **delamination**, and **epiboly**—described in **TABLE 1.1**.

In addition to establishing which cells will be in which germ layer, embryos must develop three crucial axes that are the foundation of the body: the anterior-posterior axis, the dorsal-ventral axis, and the right-left axis (**FIGURE 1.10**). The **anterior-posterior** (**AP** or **anteroposterior**) **axis** is the line extending from head to tail (or from mouth to anus in those organisms that lack a head and tail). The **dorsal-ventral** (**DV** or **dorsoventral**) **axis** is the line extending from back (dorsum) to belly (ventrum). The **right-left axis** separates the two lateral sides of the body. Although humans (for example) may look symmetrical, recall that in most of us, the heart is in the left half of the body, while the liver is on the right. Somehow, the embryo knows that some organs belong on one side and other organs go on the other.

The primary germ layers and early organs

The end of preformationism—the idea that all the organs of the adult are present in miniature in the sperm or egg (see Further Development 1.3, online)—did not come until the 1820s, when a combination of new staining techniques, improved microscopes, and institutional reforms in German universities created a revolution in descriptive embryology. The new techniques enabled microscopists to document the epigenesis of anatomical structures, and the institutional reforms provided audiences for these reports and students to carry on the work of their teachers. Among the most talented of

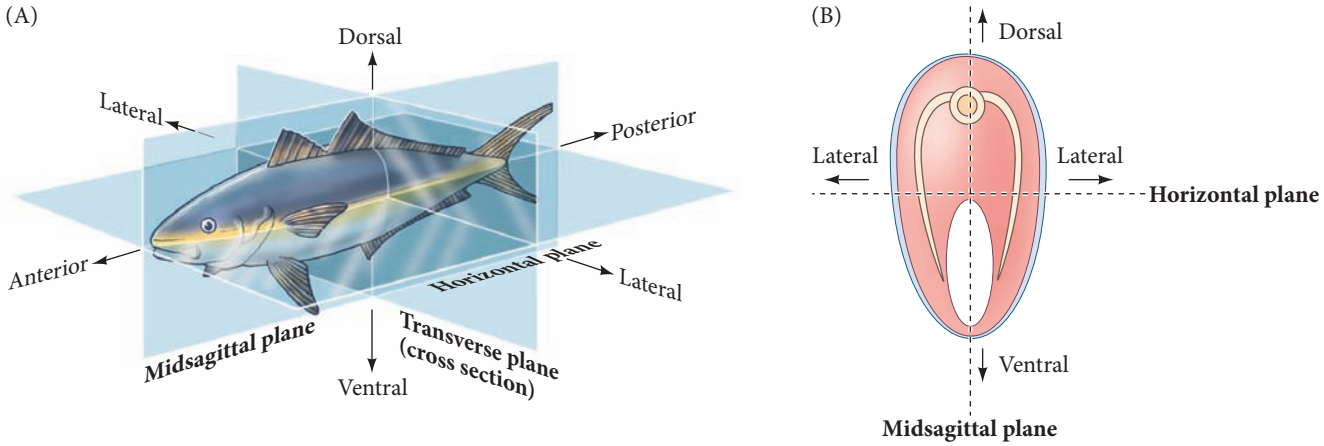


FIGURE 1.10 Axes of a bilaterally symmetrical animal. (A) A single plane, the midsagittal plane, divides the animal into left and right halves. (B) Cross sections bisecting the anterior-posterior axis.

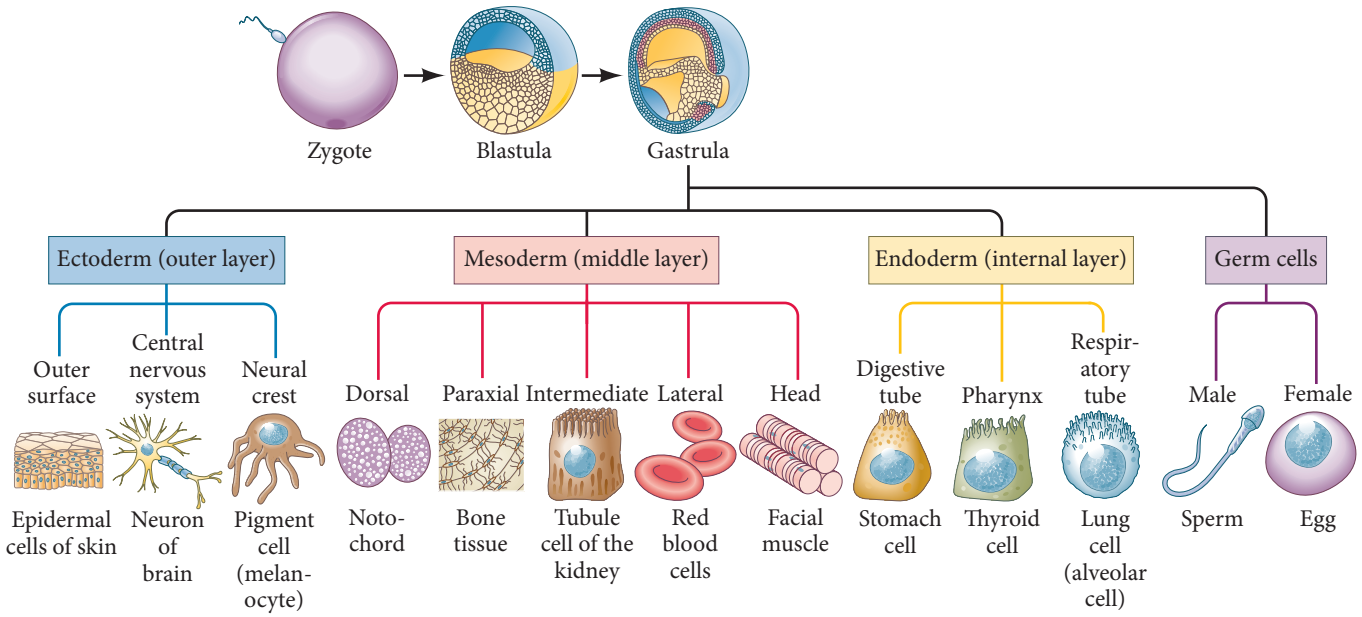


FIGURE 1.11 The dividing cells of the fertilized egg form three distinct embryonic germ layers. Each of the germ layers gives rise to myriad differentiated cell types (only a few representatives are shown here) and distinct organ systems. The germ cells (precursors of the sperm and egg) are set aside early in development and do not arise from any particular germ layer.

this new group of microscopically inclined investigators were three friends, born within a year of each other, all of whom came from the Baltic region and studied in northern Germany. The work of Christian Pander, Heinrich Rathke, and Karl Ernst von Baer transformed embryology into a specialized branch of science.

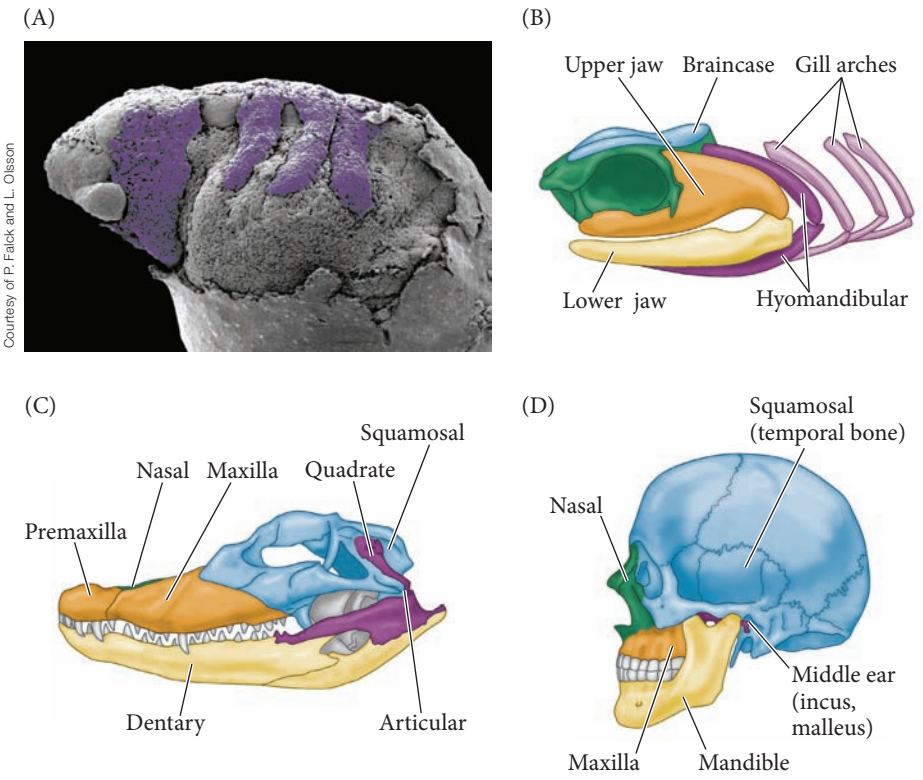
Studying the chick embryo, Pander discovered that the embryo was organized into germ layers[6]—three distinct regions of the embryo that give rise through epigenesis (i.e., forming de novo, or "from scratch") to the differentiated cell types and specific organ systems (**FIGURE 1.11**). These three layers are found in the embryos of most animal phyla:

- The ectoderm generates the outer layer of the embryo. It produces the surface layer (epidermis) of the skin and forms the brain and nervous system.
- The endoderm becomes the innermost layer of the embryo and produces the epithelium of the digestive tube and its associated organs (including the lungs).
- The mesoderm becomes sandwiched between the ectoderm and endoderm. It generates the blood, heart, kidney, gonads, bones, muscles, and connective tissues.

Pander also demonstrated that the germ layers did not form their respective organs autonomously (Pander 1817). Rather, each germ layer "is not yet independent enough to indicate what it truly is; it still needs the help of its sister travelers, and therefore, although already designated for different ends, all three influence each other collectively until each has reached an appropriate level." Pander had discovered the tissue interactions that we now call induction. No vertebrate tissue is able to construct organs by itself; it must interact with other tissues, as we will describe in Chapter 4.

Meanwhile, Rathke followed the intricate development of the vertebrate skull, excretory systems, and respiratory systems, showing that these became increasingly complex. He also showed that their complexity took on different trajectories in different classes of vertebrates. For instance, Rathke was the first to identify the **pharyngeal arches** (**FIGURE 1.12**). He showed that these same embryonic structures became gill supports in fish, and the jaws and ears (among other things) in mammals. Interestingly, the pharyngeal arches are derived from a migrating stem cell population called **neural crest cells**. Strikingly, neural crest cells break free of the

[6] From the same root as "germination," the Latin *germen* means "sprout" or "bud." The names of the three germ layers are from the Greek: ectoderm from *ektos* ("outside") plus *derma* ("skin"); mesoderm from *mesos* ("middle"); and endoderm from *endon* ("within").



Courtesy of P. Falck and L. Olsson

FIGURE 1.12 Evolution of pharyngeal arch structures in the vertebrate head. (A) Pharyngeal arches (also called branchial arches) in the embryo of the Mexican salamander, *Ambystoma mexicanum*. The surface ectoderm has been removed to permit visualization of the arches (highlighted in color) as they form from neural crest cells streaming down from the midline. (B) In adult fish, pharyngeal arch cells form the hyomandibular jaws and gill arches. (C) In amphibians, birds, and reptiles (a crocodile is shown here), these same cells form the quadrate bone of the upper jaw and the articular bone of the lower jaw. (D) In mammals, the quadrate has become internalized and forms the incus of the middle ear. The articular bone retains its contact with the quadrate, becoming the malleus of the middle ear. Thus, the cells that form gill supports in fish form the middle ear bones in mammals. (B, after R. Zangerl and M. E. Williams. 1975. *Paleontology* 18: 333–341.)

dorsal neural tube and migrate as streams of cells into a variety of peripheral parts of the head and body (see Figure 1.12A), where they give rise to such diverse cell types as cartilage and bone in the head, sensory neurons and glial cells in the body, and pigment throughout. For this extreme stem-cell-like behavior, neural crest cells are often colloquially referred to as the fourth germ layer. (See **Further Development 1.3, Epigenesis and Preformationism**, online.)

Understanding cell behavior in the embryo

By the late 1800s, it had been conclusively demonstrated that the cell is the basic unit of all anatomy and physiology. Embryologists, too, began to base their field on the cell. But unlike those who studied the adult organism, developmental anatomists found that cells in the embryo do not "stay put." Indeed, one of the most important conclusions of developmental anatomists is that embryonic cells do not remain in one place, nor do they keep the same shape (Larsen and McLaughlin 1987).

There are two major types of cells in the animal embryo: **epithelial cells**, which are tightly connected to one another in sheets or tubes; and **mesenchymal cells**, which are unconnected or loosely connected to one another and can operate as independent units. Within these two types of arrangements, morphogenesis is brought about through a limited repertoire of variations in cellular processes:

- *Direction and number of cell divisions.* Think of the faces of two dog breeds—say, a German shepherd and a poodle. The faces are made from the same cell types, but the number and orientation of the cell divisions are different (Schoenebeck et al. 2012). Think also of the legs of a German shepherd compared with those of a dachshund. The skeleton-forming cells of the dachshund have undergone fewer cell divisions than those of taller dogs. And the extreme example is all plants. Their morphological diversity is highly determined by control over cell division patterns.
- *Cell shape changes.* Cell shape change is a critical feature of animal development. Changing the shapes of epithelial cells often creates tubes out of sheets (as when the neural tube forms), and a shape change from epithelial to

mesenchymal is critical when individual cells migrate away from the epithelial sheet (as when muscle cells are formed). (As we will see in Chapter 24, this same type of epithelial-to-mesenchymal change operates in cancer, allowing cancer cells to migrate and spread from the primary tumor to new sites.) To be clear, mesenchymal cells are not present in plants, therefore neither are any of the behaviors they exhibit—namely migration.

- *Cell migration.* Cells have to move in order to get to their appropriate locations. For instance, the germ cells have to migrate into the developing gonad, and the primordial heart cells meet in the middle of the vertebrate neck and then migrate to the left part of the chest.
- *Cell growth.* Cells can change in size. This is most apparent in the germ cells: the sperm eliminates most of its cytoplasm and becomes smaller, whereas the developing egg conserves and adds cytoplasm, becoming comparatively huge. Many cells undergo an asymmetrical cell division that produces one big cell and one small cell, each of which may have a completely different fate. Here again, plants have monopolized this cellular mechanism of unidirectional growth to help elongate the vascular cell types, xylem and phloem.
- *Cell death.* Death is a critical part of life. The embryonic cells that constitute the webbing between our toes and fingers die before we are born. So do the cells of our tails. The orifices of our mouth, anus, and reproductive glands all form through **apoptosis**—the programmed death of certain cells at particular times and places. The sieve elements that make up the major conduits of the xylem in a plant are just skeletal remains of a cell wall following targeted apoptosis.
- *Changes in the composition of the cell membrane or secreted products.* Cell membranes and secreted cell products influence the behavior of neighboring cells. For instance, extracellular matrices secreted by one set of cells will allow the migration of their neighboring cells. Extracellular matrices made by other cell types will *prohibit* the migration of the same set of cells. In this way, "paths and guide rails" are established for migrating cells.

A Basic Approach to Watch Development

Approaching the bench: Find it, lose it, move it

As Dr. Viktor Hamburger once said, "Our real teacher has been and still is the embryo, who is, incidentally, the only teacher who is always right" (Holtfreter 1968). Hamburger was a developmental biologist who contributed to the creation of the entire chick embryo staging series used today (the Hamburger-Hamilton stages, or HH), an achievement that would have been impossible without careful observation and experimentation on the embryo.

How does the vertebrate brain develop such a precise network of connections? How are the carpels, stamens, and petals of a flower so perfectly organized in a radial distribution? Do the microbes residing in the gut influence the rate of intestinal stem cell division and differentiation, and if so, can that lead to cancer? Whatever the research question might be, developmental biologists have often approached the experimental design with a common mantra: find it, lose it, move it (Adams 2003). Admittedly, this is an oversimplification of the incredible variety of ways in which scientists have interrogated the mechanisms of developmental biology, but it is useful as an introduction to this field.

Find it: To study development, one needs to be able to see the subject in question. This could be the whole embryo, which is a different challenge depending on the species. Compare the access one has to a frog or zebrafish embryo that develops outside the mother with the access one has to a mouse that develops in utero or to a chick within the eggshell. Additionally, seeing things may mean observing select tissues or even individual cells within those tissues or, smaller still, the location of proteins and RNA transcripts. Advances in labeling techniques and innovations

in microscopy are continually improving how scientists can watch development, because after all, it is a process that occurs over time. However, just seeing structures and morphogenetic events provides researchers with descriptive and correlative information about a given process. Developmental biologists need to manipulate development to get at causation.

Move it (or) Lose it: Let's ponder the fascinating phenomenon that some animals, such as the Mexican salamander, can regenerate whole limbs following their amputation. Upon amputation, one of the first structures to form is the **blastema**, which includes a small bulge of proliferating cells and the epidermal cover overlying the wound. If the blastema is removed following an amputation (lose it), regeneration does not occur. In contrast, you learn something fundamentally different when you transplant a blastema to somewhere else on the salamander (move it), say someplace weird, like its back or even its eye. As a result of this transplantation, the blastema grows a limb out of that foreign location corresponding to the handedness of the body side it originated from. Yes, you will see some crazy stuff in the pages of this book! Development is, if anything, fun. But we digress. The "lose it" experiment tells you whether that thing now lost (tissue, cells, genes, etc.) was *necessary* for a given process, whereas the "move it" experiment tells you whether that thing is *sufficient*. In this example, the blastema is both necessary (required) and sufficient for limb regeneration. When the homologous gene for Pax6 is lost in a fly or mouse, the eye does not form. However, when mouse *Pax6* is transcribed in the leg of a fly… you guessed it, a fly eye forms on the fly's leg (see Figure 25.3C)! Craziness—but craziness that can be explained, and will be, in the coming chapters.

Direct observation of living embryos

Some embryos have relatively few cells, and the cytoplasms of their early blastomeres have differently colored pigments. In such fortunate cases, it is actually possible to look through the microscope and trace the descendants of a particular cell into the organs they generate. This creates a **fate map**—a diagram that "maps" larval or adult structures onto the region of the embryo from which they arose. E. G. Conklin did this by patiently following the fates of each early cell of the tunicate *Styela partita* (**FIGURE 1.13**; Conklin 1905). The muscle-forming cells of this tunicate embryo always had a yellow color, derived from a region of cytoplasm found in one particular pair of blastomeres at the 8-cell sage. Removal of this pair of blastomeres (which according to Conklin's fate map should produce the tail musculature) in fact resulted in larvae with no tail muscles, thus confirming Conklin's map (Reverberi and Minganti 1946). (See **Further Development 1.4, Conklin's Art and Science**, online.)

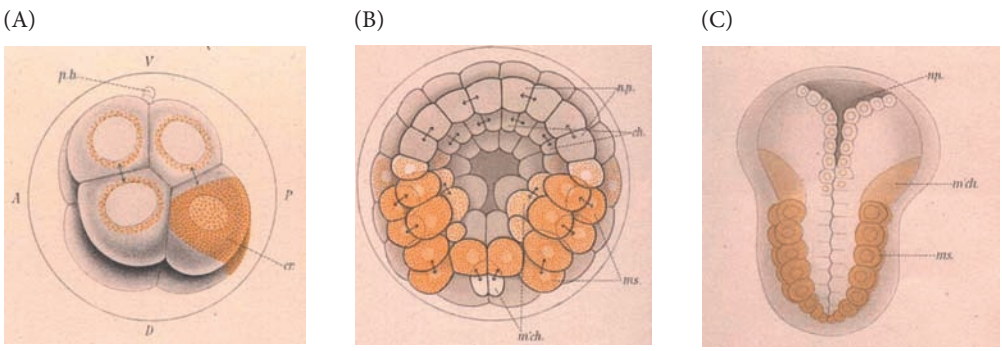


FIGURE 1.13 The fates of individual cells. Edwin Conklin mapped the fates of early cells of the tunicate *Styela partita*, using the fact that in embryos of this species, many of the cells can be identified by their different-colored cytoplasms. Yellow cytoplasm marks the cells that form the trunk muscles. (A) At the 8-cell stage, two of the eight blastomeres contain this yellow cytoplasm. (B) Early gastrula stage, showing the yellow cytoplasm in the precursors of the trunk musculature. (C) Early larval stage, showing the yellow cytoplasm in the newly formed trunk muscles. (From E. G. Conklin. 1905. *J Acad Nat Sci Phila* 13: 1–119.)

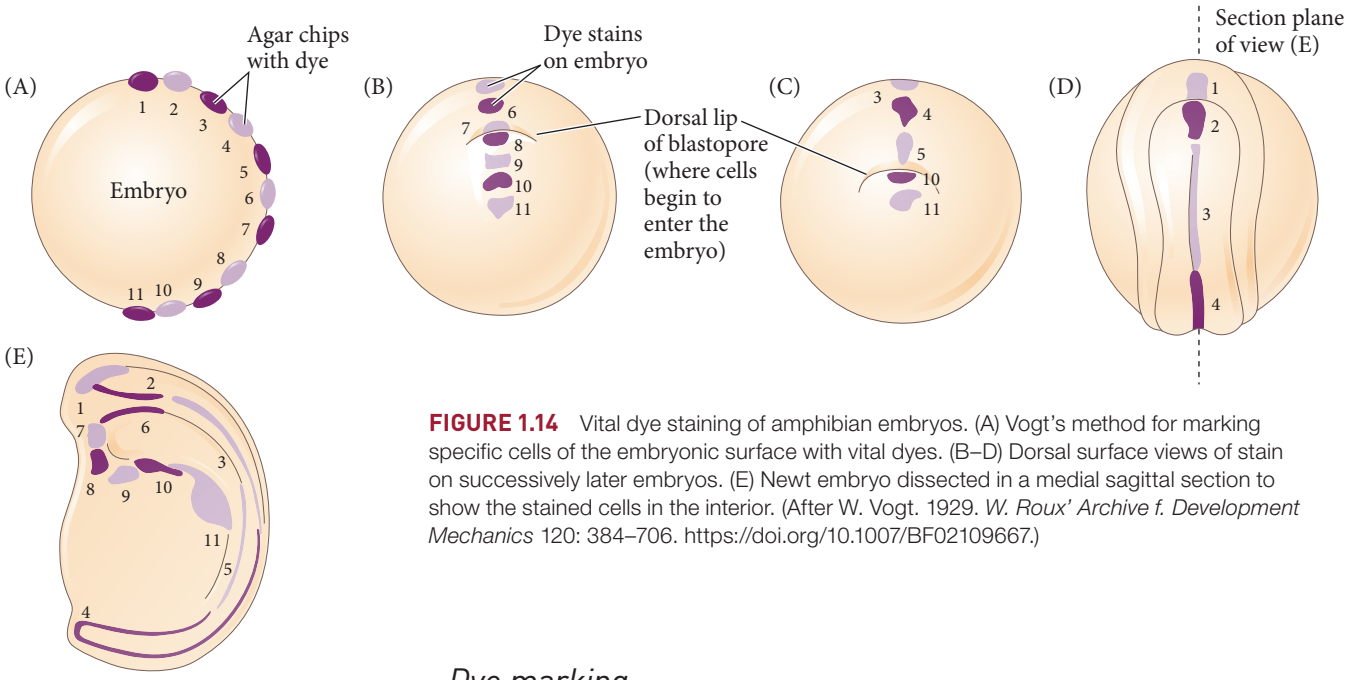


FIGURE 1.14 Vital dye staining of amphibian embryos. (A) Vogt's method for marking specific cells of the embryonic surface with vital dyes. (B–D) Dorsal surface views of stain on successively later embryos. (E) Newt embryo dissected in a medial sagittal section to show the stained cells in the interior. (After W. Vogt. 1929. *W. Roux' Archive f. Development Mechanics* 120: 384–706. https://doi.org/10.1007/BF02109667.)

Developing Questions

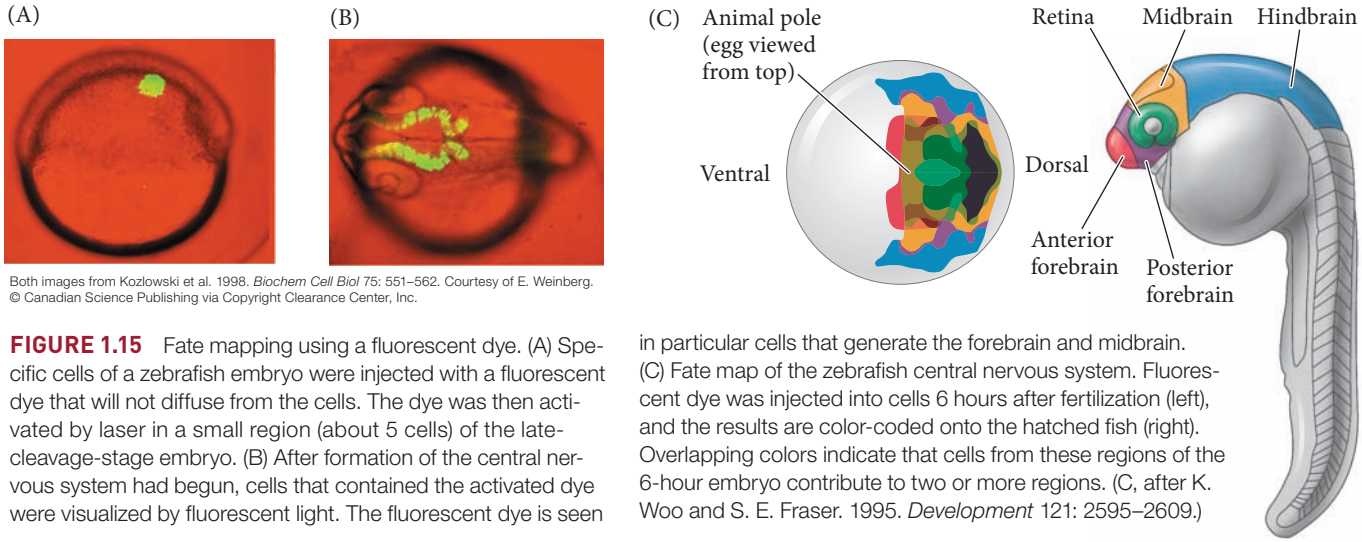
Quiz yourself: What type of experiment did Reverberi and Minganti do? Find it, lose it, or move it? As a foreshadowing question for Chapter 2, what do their results say about those yellow blastomeres?*

*Answers to Developing Questions quiz: Lose it. The results suggest that the yellow blastomeres are the only determined cells to become muscle and that they must have some sort of muscle factor that other blastomeres lack. More will be revealed in Chapter 2.

Dye marking

Most embryos are not so accommodating as to have cells of different colors. In the early years of the twentieth century, Vogt (1929) traced the fates of different areas of amphibian eggs by applying **vital dyes** to the region of interest. Vital dyes stain cells but do not kill them. Vogt mixed such dyes with agar and spread the agar on a microscope slide to dry. The ends of the dyed agar were very thin. Vogt cut chips from these ends and placed them on a frog embryo. After the dye stained the cells, he removed the agar chips and could follow the stained cells' movements within the embryo (**FIGURE 1.14**).

One problem with vital dyes is that they become more diluted with each cell division and thus over time become difficult to detect. One way around this is to use **fluorescent dyes** that are so intense that once injected into individual cells, they can still be detected in the progeny of these cells many divisions later. Fluorescein-conjugated dextran, for example, can be injected into a single cell of an early embryo, and the descendants of that cell can be seen by examining the embryo under ultraviolet light (**FIGURE 1.15**).



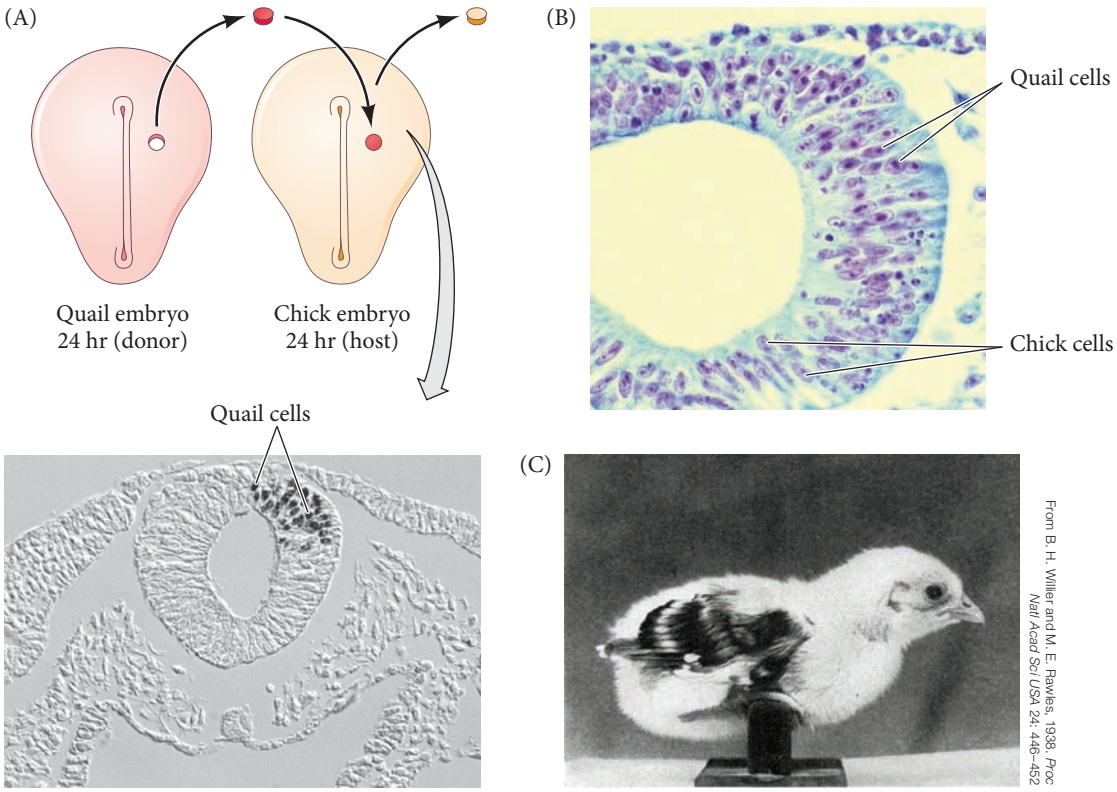
Both images from Kozlowski et al. 1998. *Biochem Cell Biol* 75: 551–562. Courtesy of E. Weinberg. © Canadian Science Publishing via Copyright Clearance Center, Inc.

FIGURE 1.15 Fate mapping using a fluorescent dye. (A) Specific cells of a zebrafish embryo were injected with a fluorescent dye that will not diffuse from the cells. The dye was then activated by laser in a small region (about 5 cells) of the late-cleavage-stage embryo. (B) After formation of the central nervous system had begun, cells that contained the activated dye were visualized by fluorescent light. The fluorescent dye is seen in particular cells that generate the forebrain and midbrain. (C) Fate map of the zebrafish central nervous system. Fluorescent dye was injected into cells 6 hours after fertilization (left), and the results are color-coded onto the hatched fish (right). Overlapping colors indicate that cells from these regions of the 6-hour embryo contribute to two or more regions. (C, after K. Woo and S. E. Fraser. 1995. *Development* 121: 2595–2609.)

Genetic labeling

One way of permanently marking cells and following their fates is to create embryos in which the same organism contains cells with different genetic constitutions. One of the best examples of this technique is the construction of **chimeric embryos**—embryos made from tissues of more than one genetic source. Chick-quail chimeras, for example, are made by grafting embryonic quail cells inside a chick embryo while the chick is still in the egg. Chick and quail embryos develop in a similar manner (especially during the early stages), and the grafted quail cells become integrated into the chick embryo and participate in the construction of the various organs (**FIGURE 1.16A**). The chick that hatches will have quail cells in particular sites, depending on where the graft was placed. Quail cells also *differ* from chick cells in several important ways, including the species-specific proteins that form the immune system. There are quail-specific proteins that can be used to find individual quail cells, even when they are "hidden" within a large population of chick cells (**FIGURE 1.16B**). By seeing where these cells migrate, researchers have been able to produce fine-structure maps of the chick brain and skeletal system (Le Douarin 1969; Le Douarin and Teillet 1973).

Chimeras dramatically confirmed the extensive migrations of the neural crest cells during vertebrate development. Mary Rawles (1940) showed that the pigment cells (melanocytes) of the chick originate in the **neural crest**, a transient band of cells that joins the neural tube to the epidermis. When she transplanted small regions of tissue containing neural crest from a pigmented strain of chickens into a similar position in an embryo from an unpigmented strain of chickens, the migrating pigment cells entered



Images A and B from D. K. Darnell and G. C. Schoenwolf. 1997. In *Molecular and Cellular Methods in Developmental Toxicology*, G. P. Daston (Ed.), pp. 231–272. CRC Press: Boca Raton, FL. Courtesy of the authors

From B. H. Willier and M. E. Rawles. 1938. *Proc Natl Acad Sci USA* 24: 446–452

FIGURE 1.16 Genetic markers as cell lineage tracers. (A) Experiment in which cells from a particular region of a 1-day quail embryo have been grafted into a similar region of a 1-day chick embryo. After several days, the quail cells can be seen by using an antibody to quail-specific proteins (photograph below). This region produces cells that populate the neural tube. (B) Chick and quail cells can also be distinguished by the heterochromatin of their nuclei. Quail cells have a single large nucleus (dense purple), distinguishing them from the diffuse nuclei of the chick. (C) Chick resulting from transplantation of a trunk neural crest region from an embryo of a pigmented strain of chickens into the same region of an embryo of an unpigmented strain. The neural crest cells that gave rise to the pigment migrated into the wing epidermis and feathers.

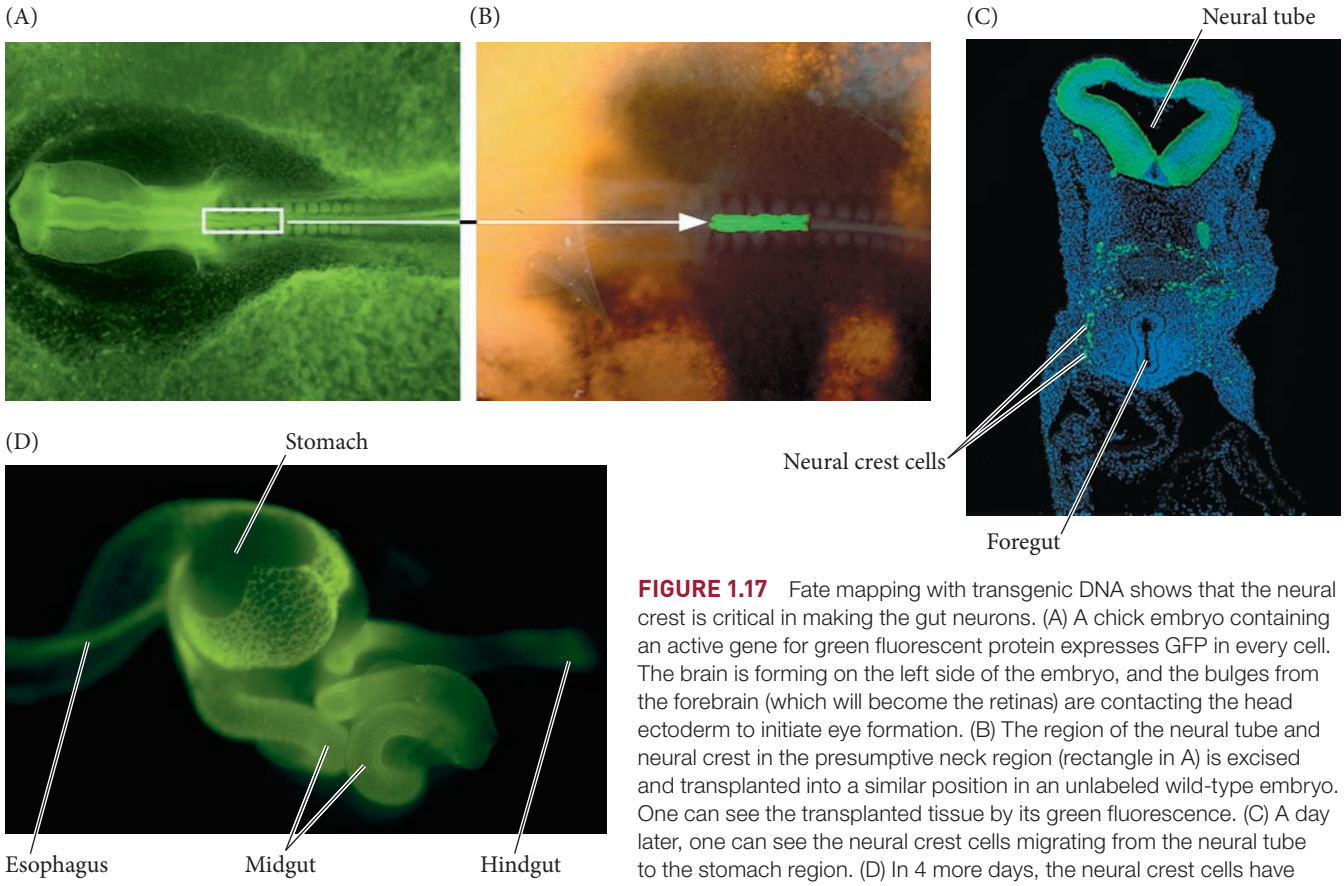
the epidermis and late entered the feathers (**FIGURE 1.16C**). Ris (1941) used similar techniques to show that, although almost all of the external pigment of the chick embryo came from the migrating neural crest cells, the pigment of the retina formed in the retina itself and was not dependent on migrating neural crest cells. This pattern was confirmed in the chick-quail chimeras, in which the quail neural crest cells produced their own pigment and pattern in the chick feathers.

Transgenic DNA chimeras

In most animals, it is difficult to meld a chimera from two species. One way to circumvent this problem is to transplant cells from a genetically modified organism. In such a technique, the genetic modification can then be traced only to those cells that express it. One version is to infect the cells of an embryo with a virus whose genes have been altered such that they express the gene for a fluorescently active protein such as **green fluorescent protein** (**GFP**).[7] A gene altered in this way is called a **transgene** because it contains DNA from another species. When the infected embryonic cells are transplanted into a wild-type host, only the donor cells and their descendants express GFP; these emit a visible green glow when placed under ultraviolet light (see Affolter 2016; Papaioannou 2016).

Variations on transgenic labeling can give us a remarkably precise map of the developing body. For example, Freem and colleagues (2012) used transgenic techniques to study the migration of neural crest cells to the gut of chick embryos, where they form the neurons that coordinate peristalsis—the muscular contractions of the gut necessary to eliminate

[7] Green fluorescent protein occurs naturally in certain jellyfish. It emits bright green fluorescence when exposed to ultraviolet light and is widely used as a transgenic label. GFP labeling will be seen in many photographs throughout this book.



All images from L. J. Freem et al. 2012. *Int J Dev Biol* 56: 245–254, courtesy of A. Burns

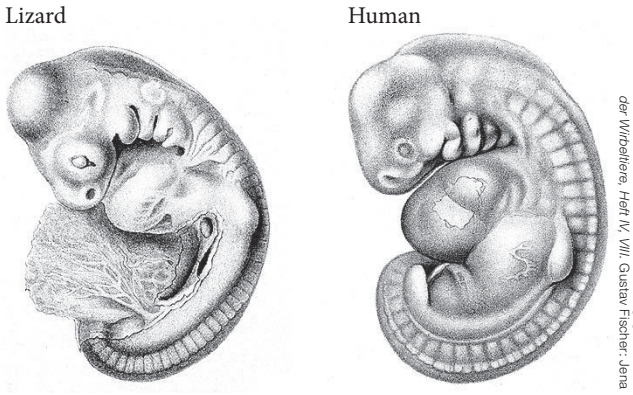
FIGURE 1.17 Fate mapping with transgenic DNA shows that the neural crest is critical in making the gut neurons. (A) A chick embryo containing an active gene for green fluorescent protein expresses GFP in every cell. The brain is forming on the left side of the embryo, and the bulges from the forebrain (which will become the retinas) are contacting the head ectoderm to initiate eye formation. (B) The region of the neural tube and neural crest in the presumptive neck region (rectangle in A) is excised and transplanted into a similar position in an unlabeled wild-type embryo. One can see the transplanted tissue by its green fluorescence. (C) A day later, one can see the neural crest cells migrating from the neural tube to the stomach region. (D) In 4 more days, the neural crest cells have spread in the gut from the esophagus to the anterior end of the hindgut.

solid waste. The parents of the GFP-labeled chick embryo were infected with a replication-deficient virus that carried an active gene for GFP. This gene was inherited by the chick embryo and expressed in every cell. In this way, Freem and colleagues generated embryos in which every cell glowed green (**FIGURE 1.17A**). They then transplanted the neural tube and neural crest of a GFP-transgenic embryo into a similar region of a normal chick embryo (**FIGURE 1.17B**). A day later, they could see GFP-labeled cells migrating into the stomach region (**FIGURE 1.17C**), and 4 days after that, the entire gut glowed green up to the anterior region of the hindgut (**FIGURE 1.17D**).

Evolutionary Embryology

"Community of embryonic structure reveals community of descent," Charles Darwin concluded in *On the Origin of Species* in 1859. This statement is based on Darwin's evolutionary interpretation of Karl Ernst von Baer's laws—namely, that relationships between groups can be established by finding common embryonic or larval forms. In 1828, just a few years before Darwin's voyage on the HMS *Beagle*, von Baer reported a curious observation. "I have two small embryos preserved in alcohol, that I forgot to label. At present I am unable to determine the genus to which they belong. They may be lizards, small birds, or even mammals." Drawings of such early-stage embryos allow us to appreciate his quandary (**FIGURE 1.18**).

From his detailed study of chick development and his comparison of chick embryos with the embryos of other vertebrates, von Baer derived four generalizations known as "von Baer's laws" (**TABLE 1.2**). Von Baer's laws can be summarized as describing how all vertebrates begin as simple embryos that share common characteristics, which become progressively specialized in species-specific ways. For instance, human embryos initially share characteristics in common with fish and avian embryos but diverge in form later in development, while never passing through the adult stages of lower vertebrate species. Recent research has confirmed von Baer's view that there is a **phylotypic stage** at which the embryos of the different groups of vertebrates all have a similar physical structure, such as the stage depicted in Figure 1.18. At this same stage there appears to be the least amount of difference among the *genes* expressed by the different groups



From F. Keibel 1904, 1908. *Normentafeln zur Entwicklungsgeschichte der Wirbeltiere, Heft IV, VIII.* Gustav Fischer: Jena

FIGURE 1.18 The vertebrates—fish, amphibians, reptiles, birds, and mammals—all start development very differently because of the enormous differences in the size of their eggs. By the beginning of neurulation, however, all vertebrate embryos have converged on a common structure. Here, a lizard embryo is shown next to a human embryo at a similar stage. As they develop beyond the neurula stage, the embryos of the different vertebrate groups become less and less like each other.

TABLE 1.2 Von Baer's laws of vertebrate embryology

1. The general features of a large group of animals appear earlier in development than do the specialized features of a smaller group.	
	All developing vertebrates appear very similar right after gastrulation. All vertebrate embryos have gill arches, a notochord, a spinal cord, and primitive kidneys. It is only later in development that the distinctive features of class, order, and finally species emerge.
2. Less general characters develop from the more general, until finally the most specialized appear.	
	All vertebrates initially have the same type of skin. Only later does the skin develop fish scales, reptilian scales, bird feathers, or the hair, claws, and nails of mammals. Similarly, the early development of limbs is essentially the same in all vertebrates. Only later do the differences between legs, wings, and arms become apparent.
3. The embryo of a given species, instead of passing through the adult stages of lower animals, departs more and more from them.	
	For example, as seen in Figure 1.12, the pharyngeal arches start off the same in all vertebrates. But the arch that becomes the jaw support in fish becomes part of the skull of reptiles and becomes part of the middle ear bones of mammals. Mammals never go through a fishlike stage (Riechert 1837; Rieppel 2011).
4. Therefore, the early embryo of a higher animal is never like a lower animal, but only like its early embryo.	
	Human embryos never pass through a stage equivalent to an adult fish or bird. Rather, human embryos initially share characteristics in common with fish and avian embryos. Later in development, the mammalian and other embryos diverge, none of them passing through the stages of the others.

within the vertebrates, indicating that this stage may be the source for the basic body plan for all the vertebrates (Irie and Kuratani 2011).[8]

After reading Johannes Müller's summary of von Baer's laws in 1842, Darwin saw that embryonic resemblances would be a strong argument in favor of the evolutionary connectedness of different animal groups. Even before Darwin, larval forms were used in taxonomic classification. In the 1830s, for instance, J. V. Thompson demonstrated that larval barnacles were almost identical to larval shrimp, and therefore he (correctly) counted barnacles as arthropods rather than mollusks (**FIGURE 1.19**; Winsor 1969). Darwin, himself an expert on barnacle taxonomy, celebrated this finding: "Even the illustrious Cuvier did not perceive that a barnacle is a crustacean, but a glance at the larva shows this in an unmistakable manner." Alexander Kowalevsky (1866) made the similar discovery that larvae of the sedentary tunicate (sea squirt) has the defining chordate structure called the notochord,[9] and that it originates from the same early embryonic tissues as the notochord does in fish and chicks. Thus, Kowalevsky reasoned, the invertebrate tunicate is related to the vertebrates, and the two great domains of the animal kingdom—invertebrates and vertebrates—are thereby united through larval structures. Darwin applauded Kowalevsky's finding, writing in *The Descent of*

[8] Indeed, one definition of a phylum is that it is a collection of species whose gene expression at the phylotypic stage is highly conserved among them, yet different from that of other species (see Levin et al. 2016). However, controversy over what constitutes a phylum persists. For instance, some authors consider cephalochordates (*Amphioxus*), tunicates, and chordates as separate phyla, whereas others unite them in one phylum, Chordata.

[9] The notochord is a rodlike structure that runs down the middle of an embryo's trunk and functions as an organizing center for the neural and non-neural tissues that surround it. It is seen in every vertebrate embryo as well as in several invertebrate embryos, including tunicates. Thus it is a defining feature of chordates (vertebrates and their invertebrate cousins—tunicates and cephalochordates, including lancelets).

(A) Barnacle

(B) Shrimp

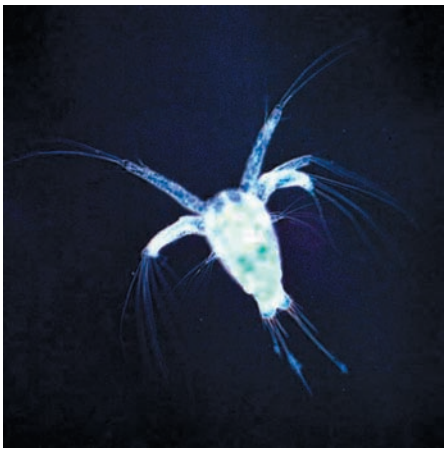


FIGURE 1.19 Larval stages reveal the common ancestry of two crustacean arthropods, barnacles (A) and shrimp (B). Barnacles and shrimp both exhibit a distinctive larval stage (the nauplius) that underscores their common ancestry as crustacean arthropods, even though adult barnacles—once classified as mollusks—are sedentary, differing in body form and lifestyle from the free-swimming adult shrimp. A larva is shown on the left in each pair of images, an adult on the right.

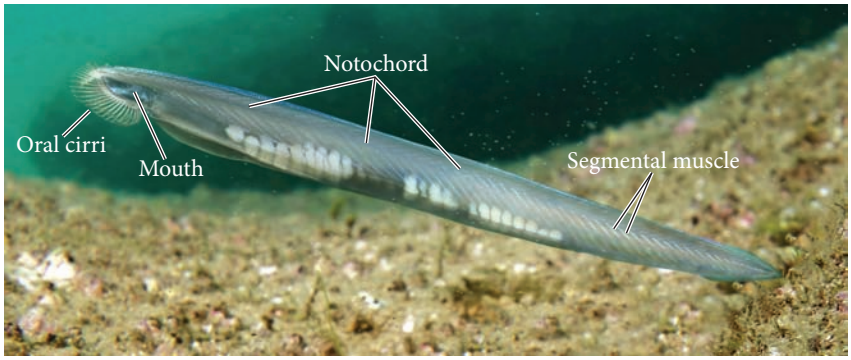
Man (1874) that "if we may rely on embryology, ever the safest guide in classification, it seems that we have at last gained a clue to the source whence the Vertebrata were derived." Darwin further noted that embryonic organisms sometimes form structures that are inappropriate for their adult form but that demonstrate their relatedness to other animals. He pointed out the existence of eyes in embryonic moles, pelvic bone rudiments in embryonic snakes, and teeth in baleen whale embryos.

FURTHER DEVELOPMENT

Chordates and the chord that connects us. Whether we are talking about an eagle, dinosaur, frog, or clownfish, they all have the common feature of being a vertebrate. The notochord is the most basal structure that defines an organism as a chordate, a group that includes the vertebrates. The notochord is a flexible rodlike structure that runs down the middle of an embryo's trunk and plays a pivotal role in organizing all surrounding tissues of the embryo. A critical moment in the transition from invertebrate to vertebrate developmental evolution is seen in *Amphioxus*, or the lancelet, a benthic, filter-feeding animal that resembles a cross between a worm and a tiny razorlike fish (**FIGURE 1.20A**). Although *Amphioxus* has no bones or even a brain of significance, it is related to the common ancestor of all chordates because it has a rudimentary notochord and nerve cord structures

FIGURE 1.20 Transitional states over the course of animal evolution. (A) *Amphioxus*, or the lancelet, has a rudimentary notochord and nerve cord structures and is thus related to the common ancestor of all vertebrates. (B) A late Jurassic (~150 mya) fossil of *Archaeopteryx* showing its distinctive features of both a reptilian skeleton and avian feathered wings. (C) *Tiktaalik roseae* emerged 375 mya from the water to be the first animal hypothesized to walk on land. This fossil (upper) and reconstruction (lower) revealed characteristics of both fish fins and amphibian forelimbs, among other characteristics. (D) Scanning electron micrograph of the cnidarian, hydra. (E) A tube sponge. Dye placed at the base of the sponge is then squirted out the top, showing the pumping action of the sponge. (F) A motile larva of a sponge.

(A)



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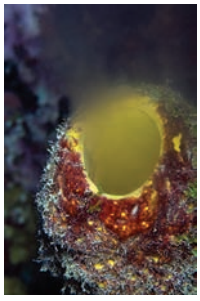

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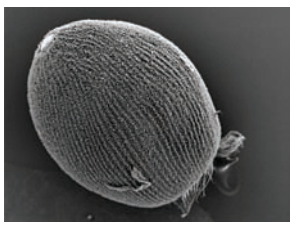

Photo courtesy of Sally Leys

(Garcia-Fernàndez and Benito-Gutiérrez 2009). This discovery, made by Alexander Kowalevsky (1867), was a milestone in biology. The developmental stages of *Amphioxus* (and tunicates) united the invertebrates and vertebrates into a single "animal kingdom."

Darwin also argued that adaptations that depart from the "type" and allow an organism to survive in its particular environment develop late in the embryo.[10] He noted that the differences among species within genera become greater as development persists, as predicted by von Baer's laws. Thus, Darwin recognized two ways of looking at "descent with modification." One could emphasize *common descent* by pointing out embryonic similarities between two or more groups of organisms, or one could emphasize the *modifications* to show how development has been altered to produce structures that enable animals and plants to adapt to particular conditions.

Understanding the tree of life to see our developmental relatedness

Earth is estimated to have formed 4.56 billion years ago (bya), with evidence of the first signs of life occurring about 3.8 bya. The theory of evolution is fundamentally based on all life on Earth originating from a common ancient ancestor, so named **LUCA**, the **last universal common ancestor**. This means all forms of life are related to one another—from you, to the elephant, to the oyster toadfish,[11] to the oyster and toad alike, to the honeybee, to the horseshoe crab, to the horrible parasitic *Ascaris* roundworm, to the beautiful brain coral, to the brain puffball mushroom, to the nearly 400,000 species of flowering plants, to the 200,000 species of protists, and even to the bacteria living in your gut. If we are all related, then the mechanisms governing how a *Homo sapiens* develops are fundamentally derived from the common ancestors that connect all life along the tree—the tree of life (**FIGURE 1.21**).

[10] As first noted by Weismann (1875), larvae must have their own adaptations. The adult viceroy butterfly mimics the monarch butterfly, but the viceroy caterpillar does not resemble the beautiful larva of the monarch. Rather, the viceroy larva escapes detection by resembling bird droppings (Begon et al. 1986).

[11] The oyster toadfish is arguably the ugliest fish in the ocean (author opinion). So yes, due to this exemplified relationship, you could consider this a personal criticism. *Yes, we are making a joke here. It's okay to laugh* (*at the joke or us—both welcomed*).

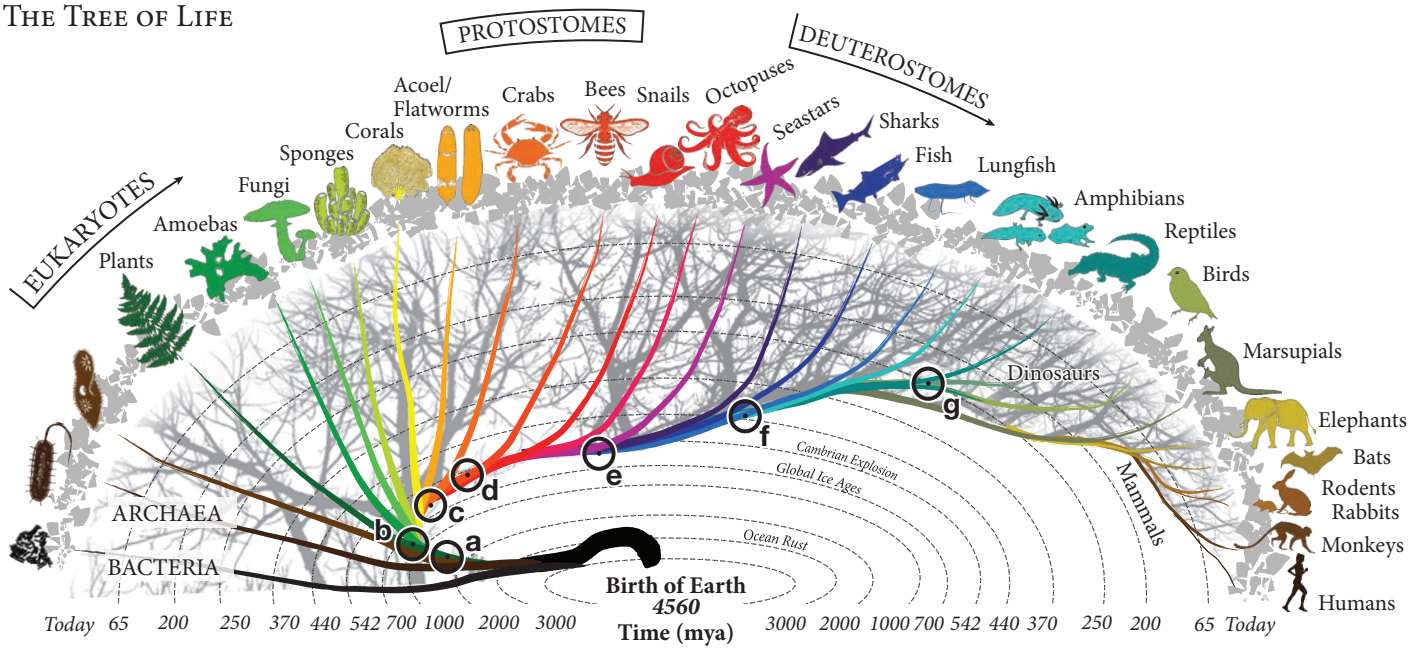

FIGURE 1.21 The tree of life—an illustration of the major branches of life. A geological timescale moves radially from the bottom to the top of the diagram. All life on Earth is related. To better comprehend this reality, some of the major organismal groups are illustrated with colored branches for simplicity. The underlying layer of gray branches implies a more realistic and chaotic interconnectedness of life's lineage. The letters a–g denote the locations of common ancestors, including those of plants (b) and of multicellular organisms (a). Many of the common ancestors of acoels and flatworms, insects, vertebrates, and land animals (annelids, arthropods, mollusks, echinoderms, and vertebrates) (c–f) can be traced to the Cambrian explosion of diversity.

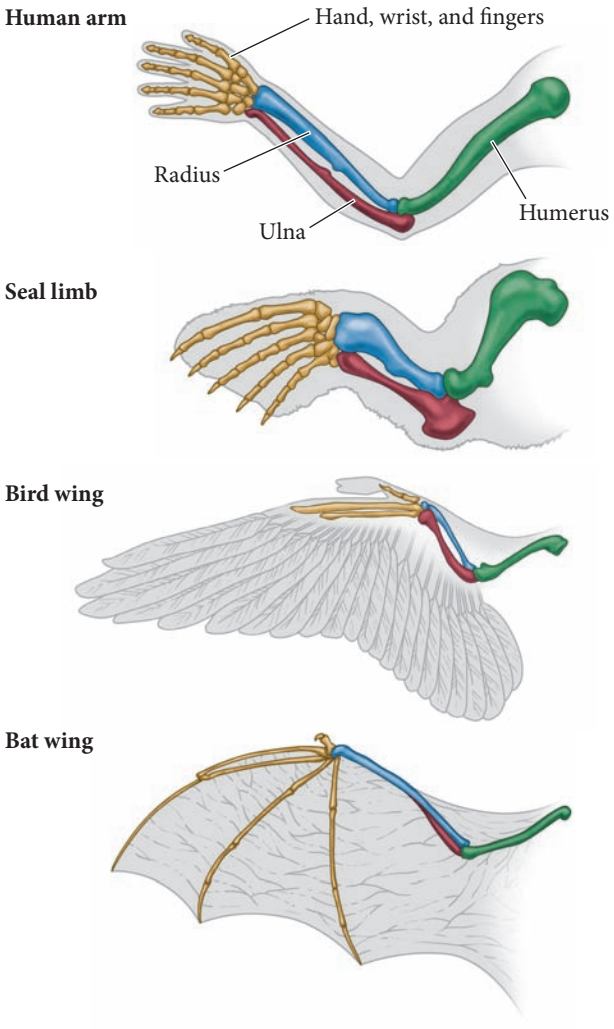

FIGURE 1.22 Homologies of structure among a human arm, a seal forelimb, a bird wing, and a bat wing; homologous supporting structures are shown in the same color. All four limbs were derived from a common tetrapod ancestor and are thus homologous as forelimbs. The adaptations of bird and bat forelimbs to flight, however, evolved independently of each other, long after the two lineages diverged from their common ancestor. Therefore, as wings they are not homologous, but analogous.

One of the most important distinctions made by evolutionary embryologists was the difference between *analogy* and *homology*. Both terms refer to structures that appear to be similar. **Homologous** structures are those whose underlying similarity arises from their being derived from a common ancestral structure. For example, the wing of a bird and the arm of a human are homologous, both having evolved from the forelimb bones of a common ancestor. Moreover, their respective parts are homologous (**FIGURE 1.22**).

Analogous structures are those whose similarity comes from their performing a similar function rather than their arising from a common ancestor. For example, the wing of a butterfly and the wing of a bird are analogous; the two share a common function (and thus both are called wings), but the bird wing and insect wing did not arise from a common ancestral structure that became modified through evolution into bird wings and butterfly wings. Homologies must always refer to the level of organization being compared. For instance, bird and bat wings are homologous as forelimbs but not as wings. In other words, they share an underlying structure of forelimb bones because birds and mammals share a common ancestor that possessed such bones. Bats, however, descended from a long line of non-winged mammals, whereas bird wings evolved independently, from the forelimbs of ancestral reptiles (follow the tree branches in Figure 1.21).

As we will see in Chapter 25, evolutionary change is based on developmental change. The bat wing, for example, is made in part by (1) maintaining a rapid growth rate in the cartilage that forms the fingers and (2) preventing the cell death that normally occurs in the webbing between the fingers. As seen in **FIGURE 1.23**, mice start off with webbing between their digits (as do humans and most other mammals). This webbing is important for creating the anatomical distinctions between the fingers. Once the webbing has served that function, genetic signals cause its cells to die, leaving free digits that can grasp and manipulate. Bats, however, use their fingers for flight, a feat accomplished by changing the expression of those genes in the cells of the webbing. The genes activated in embryonic bat webbing encode proteins that *prevent* cell death, as well as proteins that accelerate finger elongation (Cretekos et al. 2005; Sears et al. 2006; Weatherbee et al. 2006). Thus, homologous anatomical structures can differentiate by altering development, and such changes in development provide the variation needed for evolutionary change.

Charles Darwin observed artificial selection in pigeon and dog breeds, and these examples remain valuable resources for studying selectable variation. For instance, the short legs of dachshunds were selected by breeders who wanted to use these dogs to hunt badgers (German *Dachs*, "badger" + *Hund*, "dog") in their underground burrows. The mutation that causes the dachshund's short legs involves an extra copy of the *Fgf4* gene, which makes a protein that informs the cartilage precursor cells that they have divided enough and can start differentiating. With this extra copy of *Fgf4*, cartilage cells are told that they should stop dividing earlier than in most other dogs, so the legs stop growing (Parker et al. 2009). Similarly, long-haired dachshunds differ from their short-haired relatives in having a mutation in the *Fgf5* gene (Cadieu et al. 2009). This gene is involved in hair production and allows each follicle to make a longer hair shaft (Ota et al. 2002). It is the embryo where genotype is translated into phenotype, where

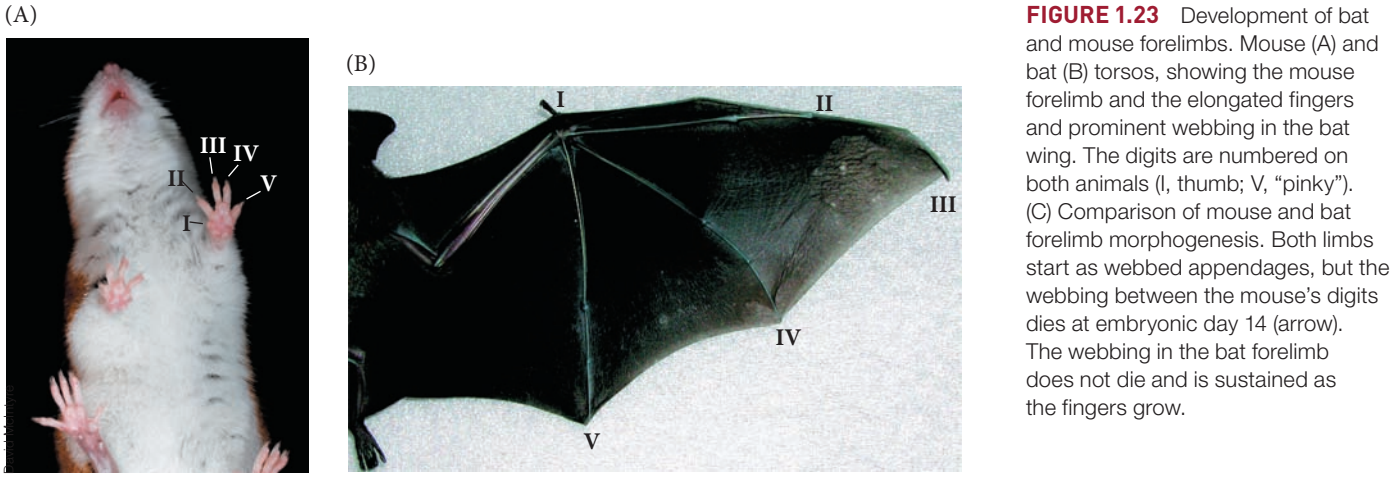

FIGURE 1.23 Development of bat and mouse forelimbs. Mouse (A) and bat (B) torsos, showing the mouse forelimb and the elongated fingers and prominent webbing in the bat wing. The digits are numbered on both animals (I, thumb; V, "pinky"). (C) Comparison of mouse and bat forelimb morphogenesis. Both limbs start as webbed appendages, but the webbing between the mouse's digits dies at embryonic day 14 (arrow). The webbing in the bat forelimb does not die and is sustained as the fingers grow.

B and C from C. J. Cretekos et al. 2008. *Genes Dev* 22: 141–151, courtesy of C. J. Cretekos © Cold Spring Harbor Laboratory Press

inherited genes are expressed to form the adult. Thus, mutations in genes controlling developmental processes can generate selectable variation.

KEY EMBRYONIC TRANSITIONS IN ANIMALS OVER EVOLUTIONARY HISTORY How do we know that one animal form actually preceded the evolution of another form? It's not as if we can literally see a lizard suddenly sprout feathers on its forelimbs and fly off into the sky. However, there are examples of some creatures showing traits of two closely related species, a so-called transitional morphological state. By examining such transitional organisms over the evolutionary history of metazoans (all animals), we can illuminate some important aspects of embryonic development that were altered to drive the morphological diversity we see today. For instance, the fossil record has revealed combined features of fin and leg in *Tiktaalik roseae*, suggesting it was the first aquatic species to walk on land. Similarly, fossils of the dinosaur *Archaeopteryx* possess a reptilian skeleton with feathered wings, showing the evolutionary relatedness between dinosaur and bird and the morphological transition from one to the other (**FIGURE 1.20B,C**; see also review by Stefan Rensing 2016).

SCIENTISTS SPEAK 1.1 "Your Inner Fish" by Neil Shubin.

FURTHER DEVELOPMENT

THE ORIGINS OF BILATERAL SYMMETRY AND OUR THREE EMBRYONIC GERM LAYERS Bilateral symmetry found in most animal groups is thought to have evolved from organisms possessing simpler radial and spherical geometric morphologies, as we see in today's cnidarians (jellyfishes, corals, hydra, and their