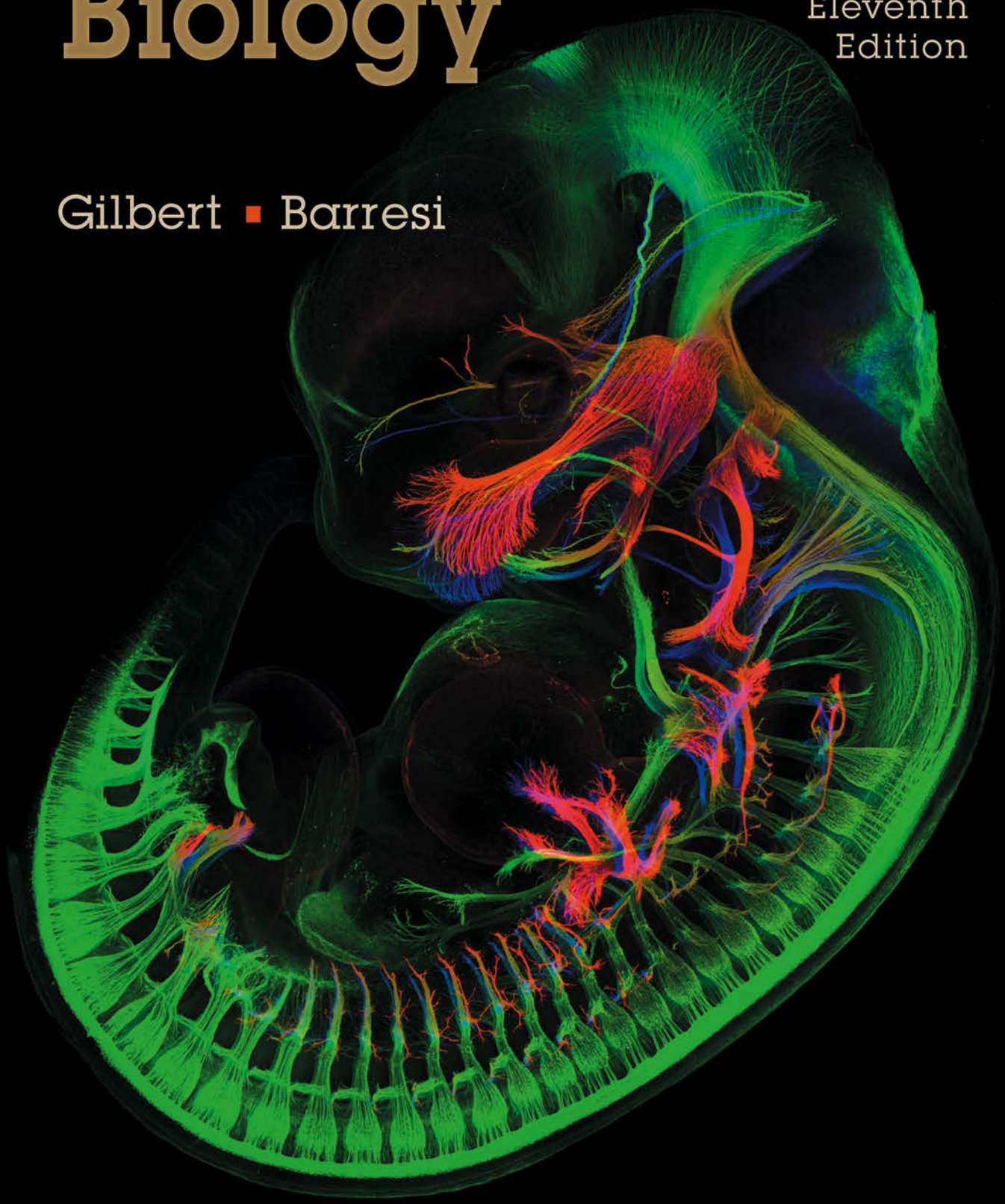
Developmental Biology

Eleventh Edition

Gilbert ▪ Barresi



INCLUDED WITH THIS BOOK

DevBio Laboratory: Vade Mecum³

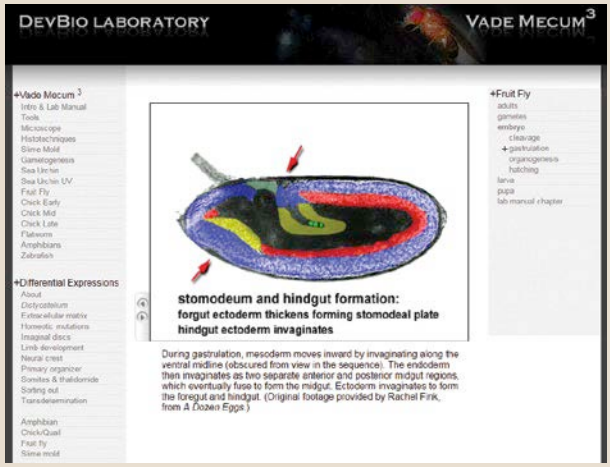
An Interactive Guide to Developmental Biology

Mary S. Tyler and Ronald N. Kozlowski

labs.devbio.com

Designed to complement the textbook, this unique resource helps you understand the organisms discussed in lecture and prepares you for the laboratory. ***DevBio Laboratory: Vade Mecum³*** is available online, which allows you the flexibility to use the software from any computer or mobile device.

Over 140 interactive videos and 300 labeled photographs take you through the life cycles of model organisms used in developmental biology laboratories. The easy-to-use videos provide you with the concepts, vocabulary, and motivation to enter the laboratory fully prepared. A chapter on zebrafish addresses how to raise the organism and the effects of various teratogens on embryonic development. The site also includes chapters on: the slime mold *Dictyostelium discoideum*; planarian; sea urchin; the fruit fly *Drosophila melanogaster*; chick; and amphibian.



ADDITIONAL FEATURES INCLUDE:

- **Movie excerpts** from ***Differential Expressions²***: Short video excerpts about key concepts in development
- **PowerPoint® slides** of chick wholemounts and serial sections for self-quizzing and creating tests
- **Full video instruction** on histological techniques
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- **Laboratory Manual:** The Third Edition of Mary S. Tyler's laboratory manual, ***Developmental Biology: A Guide for Experimental Study***, designed for use with the multimedia chapters of ***DevBio Laboratory: Vade Mecum³***
- **Study Questions**
- **Laboratory Skills Guides**

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YOUR GUIDE TO

Developmental Biology

9

The Genetics of Axis Specification in *Drosophila*

Opening Question and Photo

Each chapter begins with an intriguing question and photo, encouraging your learning and discovery as the concepts unfold.

THANKS LARGELY TO STUDIES spearheaded by Thomas Hunt Morgan's laboratory during the first two decades of the twentieth century, we know more about the genetics of *Drosophila melanogaster* than that of any other multicellular organism. The reasons have to do with both the flies themselves and with the people who first studied them. *Drosophila* is easy to breed, hardy, prolific, and tolerant of diverse conditions. Moreover, in some larval cells, the DNA replicates several times without separating. This leaves hundreds of strands of DNA adjacent to each other, forming polytene (Greek, "many strands") chromosomes (**FIGURE 9.1**). The unused DNA is more condensed and stains darker than the regions of active DNA. The banding patterns were used to indicate the physical location of the genes on the chromosomes. Morgan's laboratory established a database of mutant strains, as well as an exchange network whereby any laboratory could obtain them.

Historian Robert Kohler noted in 1994 that "The chief advantage of *Drosophila* initially was one that historians have overlooked: it was an excellent organism for student projects." Indeed, undergraduates (starting with Calvin Bridges and Alfred Sturtevant) played important roles in *Drosophila* research. The *Drosophila* genetics program, says Kohler, was "designed by young persons to be a young person's game," and the students set the rules for *Drosophila* research: "No trade secrets, no monopolies, no p

Jack Schultz (originally in Morgan's laboratory) and other
burgeoning supply of data on the genetics of *Drosophila* to its dev
was a difficult organism on which to study embryology. Fly e
and intractable, being neither large enough to manipulate experi

The Punchline

Here you will be clearly and quickly guided toward the big principles that will be exemplified in the chapter.

What changes in development caused this fly to have four wings instead of two?

The Punchline

The development of the fruit fly is extremely rapid, and its body axes are specified by factors in the maternal cytoplasm even before the sperm enters the egg. The anterior-posterior axis is specified by proteins and mRNAs made in maternal nurse cells and transported into the oocyte, such that each region of the egg contains different ratios of anterior- and posterior-promoting proteins. Eventually, gradients of these proteins control a set of transcription factors—the homeotic proteins—that specify the structures to be formed by each segment of the adult fly. The dorsal-ventral axis is also initiated in the egg, which sends a signal to its surrounding follicle cells. The follicle cells respond by initiating a molecular cascade that leads both to cell-type specification and to gastrulation. Specific organs form at the intersection of the anterior-posterior axis and the dorsal-ventral axis.

Scientists Speak

In these interviews, emerging topics in developmental biology are discussed by leading experts in the field.

Web Topic

Here you are provided with more information about cutting-edge topics, as well as historical, philosophical, and ethical perspectives, in addition to links to online resources.

Next Step Investigation

This feature provides insights into some of the field's greatest challenges, inspiring curiosity and further exploration.

Closing Thoughts on the Opening Photo

Coming full circle, this feature relates chapter concepts back to the Opening Question and Photo.

Developing Questions

These questions are an entry-way for independent research, empowering you to expand your knowledge and enhance your participation in class discussion.

Snapshot Summary

This closing feature provides you with a step-by-step breakdown of the chapter text.

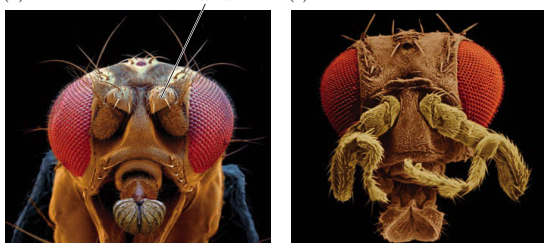


FIGURE 9.26 (A) Head of a wild-type fruit fly. (B) Head of a fly containing the *Antennapedia* mutation that converts antennae into legs. (A © Eye of Science/ Science Source; B © Science VU/Dr. F. Rudolph Turner/Visuals Unlimited, Inc.)

can be detected in specific regions of the embryo (see Figure 9.24B) and are especially prominent in the central nervous system.

SCIENTISTS SPEAK 9.3 Listen to this interview with Dr. Walter Gehring, who spearheaded investigations that unified genetics, development, and evolution, leading to the discovery of the homeobox and its ubiquity throughout the animal kingdom.

WEB TOPIC 9.5 **INITIATION AND MAINTENANCE OF HOMEOTIC GENE EXPRESSION** Homeotic genes make specific boundaries in the *Drosophila* embryo. Moreover, the protein products of the homeotic genes activate batteries of other genes, specifying the segment.

Developing Questions

Homeobox genes specify the anterior-posterior body axis in both *Drosophila* and humans. How come we do not see homeotic mutations that result in extra sets of limbs in humans, as can happen in flies?

Generating the Dorsal-Ventral Axis

Dorsal-ventral patterning in the oocyte

As oocyte volume increases, the oocyte nucleus is pushed by the growir to an anterior dorsal position (Zhao et al. 2012). Here the *gurken* mess been critical in establishing the anterior-posterior axis, initiates the fo

Next Step Investigation

The precision of *Drosophila* transcription patterning is remarkable, and a transcription factor may specify whole regions or small parts. Some of the most important regulatory genes in *Drosophila*, such as the gap genes, have been found to have "shadow enhancers," secondary enhancers that may be quite distant from the gene. These shadow enhancers seem to be critical for the fine-tuning of gene expression, and they may cooperate or compete with the main enhancer. Some of these shadow enhancers may work under particular physiological stresses. New studies are showing that the robust phenotypes of flies may result from an entire series of secondary enhancers that are able to improvise for different conditions (Bothma et al. 2015).

Closing Thoughts on the Opening Photo

In the fruit fly, inherited genes produce proteins that interact to specify the normal orientation of the body, with the head at one end and the tail at the other. As you studied this chapter, you should have observed how these interactions result in the specification of entire blocks of the fly's body as modular units. A patterned array of homeotic proteins specifies the structures to be formed in each segment of the adult fly. Mutations in the genes for these proteins, called homeotic mutations, can change the structure specified, resulting in wings where there should have been halteres, or legs where there should have been antennae (see pp. 242–243). Remarkably, the proximal-distal orientation of the mutant appendages corresponds to the original appendage's proximal-distal axis, indicating that the appendages follow similar rules for their extension. We now know that many mutations affecting segmentation of the adult fly in fact work on the embryonic modular unit, the parasegment (see pp. 234 and 240). You should keep in mind that, in both invertebrates and vertebrates, the units of embryonic construction often are not the same units we see in the adult organism. (Photograph courtesy of Nipam Patel.)

9 Snapshot Summary

Drosophila *Development and Axis Specification*

1. *Drosophila* cleavage is superficial. The nuclei divide 13 times before forming cells. Before cell formation, the nuclei reside in a syncytial blastoderm. Each nucleus is surrounded by actin-filled cytoplasm.
2. When the cells form, the *Drosophila* embryo undergoes a mid-blastula transition, wherein the cleavages become asynchronous and new mRNA is made. At this time, there is a transfer from maternal to zygotic control of development.
3. Gastrulation begins with the invagination of the most ventral region (the presumptive mesoderm), which causes the formation of a ventral furrow. The germ band expands such that the future posterior segments curl just

- There is a *temporal order* genes are transcribed, and often regulate the expressi
- *Boundaries* of gene expres the interaction between transcription factors and their gene targets. Here, the transcription factors transcribed earlier regulate the expression of the next set of genes.
- *Translational control* is extremely important in the early embryo, and localized mRNAs are critical in patterning the embryo.
- *Individual cell fates* are not defined immediately. Rather, there is a stepwise specification wherein

All **Web Topics**, **Dev Tutorials**, **Watch Development**, and **Scientists Speak**

Vade Mecum

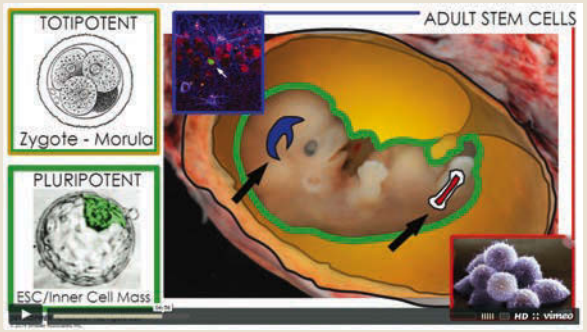
This interactive website will help you understand the organisms discussed in the course, preparing you for the lab.

Watch Development

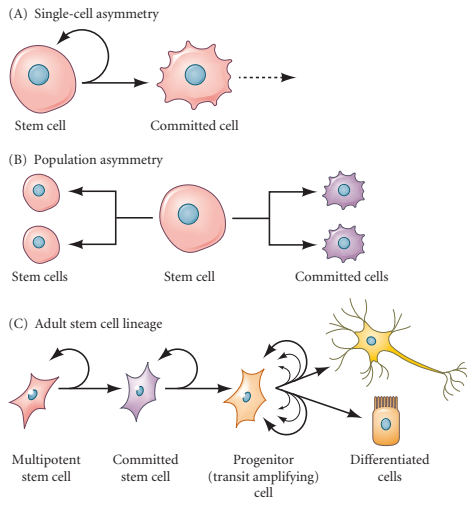
Putting concepts into action, these informative videos show you real-life developmental biology processes.

Dev Tutorial

Providing additional ways for you to explore topics, these video tutorials, presented by the book's authors, reinforce key concepts from the text.



...m cell concept. (A) The fun-
...tem cell is that it can make
...also producing cells committed
...tiation. This is called asym-
... A population of stem cells
...d through population asym-
...ll is shown to have the ability
... to produce either two stem
...he stem cell pool by 1) or to
...ed cells (thus decreasing the
...ions are termed symmetrical
...al differentiating. (C) In many
...ges pass from a multipotent
...orming numerous types of cells)
...ell that makes one or very few
...genitor (transit amplifying) cell
... multiple rounds of divisions but
...d is committed to becoming a
...rentiated cell.



...ing). Researchers are now able to identify developmental interactions taking place in very small regions of the embryo, to identify enhancers and their transcription factors, and to mathematically model the interactions to a remarkable degree of precision (Hengenius et al. 2014).

Early *Drosophila* Development

We have already discussed the specification of early embryonic cells by cytoplasmic determinants stored in the oocyte. The cell membranes that form during cleavage establish the region of cytoplasm incorporated into each new blastomere, and the morphogenetic determinants in the incorporated cytoplasm then direct differential gene expression in each cell. But in *Drosophila* development, cell membranes do not form until after the thirteenth nuclear division. Prior to this time, the dividing nuclei all share a common cytoplasm and material can diffuse throughout the whole embryo. The specification of cell types along the anterior-posterior and dorsal-ventral axes is accomplished by the interactions of components *within* the single multinucleated cell. Moreover, these axial differences are initiated at an earlier developmental stage by the position of the egg within the mother's egg chamber. Whereas the sperm entry site may fix the axes in nematodes and tunicates, the fly's anterior-posterior and dorsal-ventral axes are specified by interactions between the egg and its surrounding follicle cells prior to fertilization.

VADE MECUM
The fruit fly chapter has remarkable time-lapse sequences, including footage of cleavage and gastrulation. This chapter also provides access to the fly life cycle.

WATCH DEVELOPMENT 9.1 The website "The Interactive Fly" features movies illustrating all aspects of *Drosophila* development.

The Stem Cell Concept

Division and self-renewal

A cell is a stem cell if it can divide and in doing so produce a replica of itself (a process called **self-renewal**) as well as a daughter cell that can undergo further development. Stem cells are often referred to as undifferentiated due to this maintenance of proliferative properties[1]. Upon division, a stem cell may also produce a daughter cell that can mature into a terminally differentiated cell type. Cell division can occur either symmetrically or asymmetrically. If a stem cell divides symmetrically, it could produce two self-renewing stem cells or two daughter cells that are committed to differentiate, resulting in either the expansion or reduction of the resident stem cell population, respectively. In contrast, if the stem cell divides asymmetrically, it could stabilize the stem cell pool as well as generate a daughter cell that goes on to differentiate. This strategy, in which two types of cells (a stem cell and a developmentally committed cell) are produced at each division, is called the *single stem cell asymmetry* mode and is seen in many types of stem cells (**FIGURE 5.1A**). An alternative (but not mutually exclusive) mode of retaining cell homeostasis is the *population asymmetry* mode of stem cell division. Here, some stem cells are more prone to produce differentiated progeny, and this is compensated for by another set of stem cells that divide symmetrically to maintain the stem cell pool within this population (**FIGURE 5.1B**; Watt and Hogan 2000; Simons and Clevers 2011).

DEV TUTORIAL
Stem Cell Basics

[1]There are many different stem cells and so their status as "undifferentiated" really only pertains to the retained ability to divide, but they are in fact a defined cell type.

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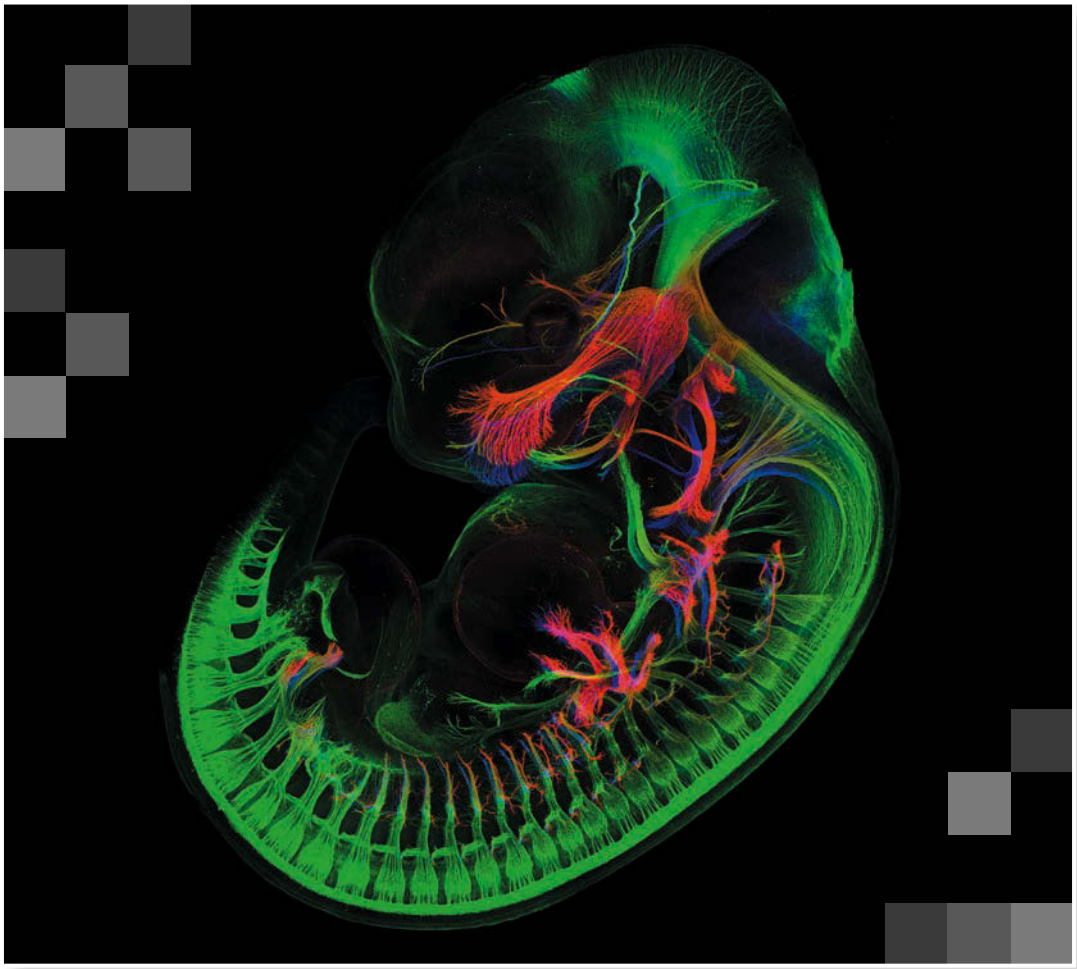


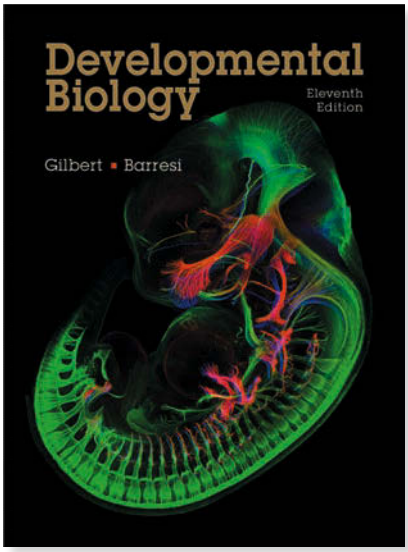
Scott F. Gilbert

Swarthmore College and the University of Helsinki

Michael J. F. Barresi

Smith College

Sinauer Associates, Inc., Publishers ■ Sunderland, Massachusetts U.S.A.



The Cover

The axons of the developing peripheral nervous system are stained red in this confocal micrograph of a whole mount mouse embryo at day 11.5 of development. The growth and specific targeting of axons during vertebrate development are discussed in Chapter 15. Photograph courtesy of Zhong Hua and Jeremy Nathans, Johns Hopkins University.

Developmental Biology, 11th Edition

For more information, address:
Sinauer Associates, Inc.
P.O. Box 407
Sunderland, MA 01375 USA
FAX 413-549-1118
publish@sinauer.com
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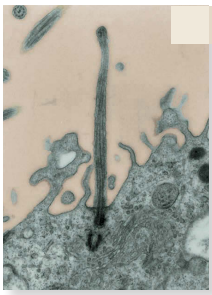
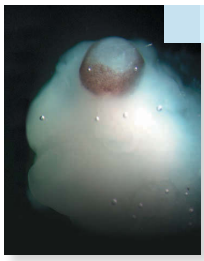
To Daniel, Sarah, David, and Natalia
S. F. G.

To Scott Gilbert
who offered me this opportunity.
&
To my family, Heather, Samuel, Jonah, Luca, and Mateo
who enabled me to take advantage of this opportunity.
M. J. F. B.

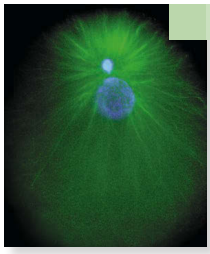
Brief Contents

Contents





PART II ■ Gametogenesis and Fertilization: The Circle of Sex



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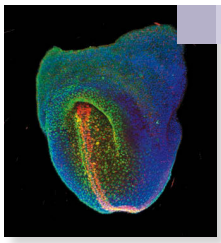

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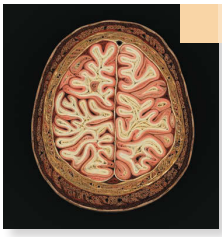
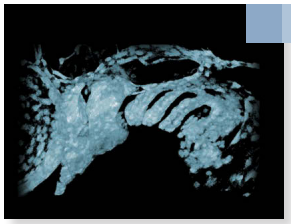
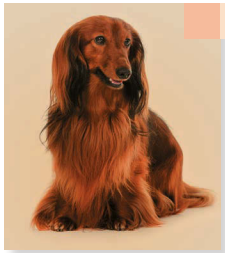
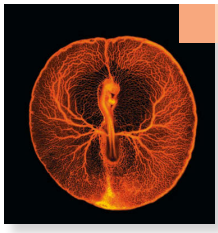
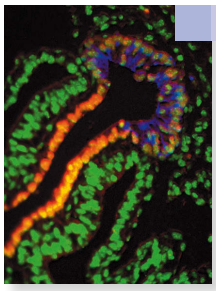


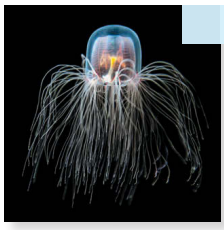




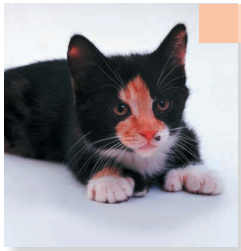


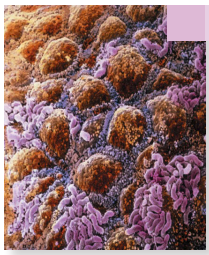


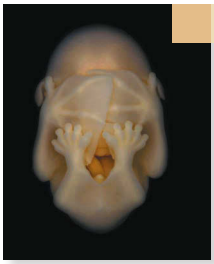
PART VI ■ Postembryonic Development



PART VII ■ Development in Wider Contexts







From the Authors

From Scott Gilbert

A BIOLOGIST, A PHILOSOPHER, AND A THEOLOGIAN WALK INTO A BAR. Yes, it actually happened, in the chill of a winter night in Finland! A group of enthusiastic people listened as the moderator asked what each of them considered to be the most important story people need to know. The Christian theologian said that the most important story was salvation through God's grace. The analytic philosopher disagreed, saying that the most important story for mankind was that of the Enlightenment. The developmental biologist knew that he was supposed to say "evolution." But evolution is the consequence of another, more fundamental story. So the biologist claimed that most inspiring and meaningful story was how the embryo constructs itself. You pass from unformed zygote to the adult organism with its heart, brain, limbs, and gut all properly differentiated and organized. It is a story of how newness is created, how one keeps one's identity while building oneself, and how global forces and local forces work together to generate a functional entity. This is the story we tell in this book.

In the Ninth and Tenth editions of *Developmental Biology*, we speculated that the study of animal development was undergoing metamorphosis. The field has not reached the climax phase yet, but certain differences between the previous edition and one in your hands (or on your screen) are definitely apparent. The first can be seen on the cover. Developmental biology has been charged with a huge undertaking—nothing less than discovering the anatomical and genetic bases of neural organization and behaviors. This task was part of developmental biology when it was reformulated in the early 1900s (especially by the American C. O. Whitman), but it had dropped out of the portfolio as being "too complicated" and not amenable for study. Today, however, developmental neurobiology is an increasingly large part of developmental biology. Among many other things, developmental biology is becoming necessary for cognitive science.

The second difference between this and previous editions is the prominence of stem cells. From being a small area of developmental biology, stem cell research has grown so fast as to have its own scientific societies. Not only do stem cells provide explanations for organ development, they also hold the tantalizing possibility of organ regeneration. Recent work, detailed in this book, shows how knowledge of developmental biology has been critical in turning adult cells into stem cells that can functionally replace missing and damaged tissue in laboratory animals.

A third difference is the incredible revolution in lineage studies made possible by in vivo labeling. We can look at each cell developing in an early, living, embryo and discern which adult cells are its descendants. The techniques of computer-enhanced visualization have given scientists amazing new technologies to see embryonic development.

A fourth difference is the idea that animal development, even that of mammals, is significantly influenced by the environment. The data that have accumulated for developmental plasticity and the roles of microbes in normal development have increased remarkably over the past several years.

Finally, a fifth difference concerns the way science is taught. The "sage on the stage" model, where lectures generate the flow of information down a gradient from higher concentration to lower, has been supplemented by the "guide on the side." Here, the professor becomes a facilitator or capacitator of discussion while the students are encouraged to discover the information for themselves.

Indeed, education is sometimes referred to as "development," and there are many similarities between education and embryology. The two fields have exchanged metaphors constantly for the past two centuries, and two German words that have been used for both development and education—*Bildung* and *Entwicklung*—connote education by experience and education by instruction, respectively. Both work in different situations. So in this edition of *Developmental Biology*, we have tried to facilitate those professors who wish to experiment with different teaching methods. As in embryology, we don't expect one method to be best for all occasions.

To all these ends, this book has metamorphosed to embrace a co-author. Michael J. F. Barresi is expert in all these areas of stem cells, developmental neurobiology, and new techniques of learning and teaching. It's been 30 years since the first edition of this book was published, and I wanted a young professor to reconfigure this book into a learning tool that a new generation of teachers could use to inspire a new generation of students. Enter Michael. Michael did not want just cosmetic changes in the book. He proposed a radical re-envisioning of its mission: to educate students to appreciate and participate in developmental biology.

Michael convinced us that we needed to rearrange the order of the chapters, add some chapters and shorten others, alter the ways that the material is presented within the chapters, and give all chapters more supplemental material for "flipped" classes, case studies, and other means of learning. The extra thought and effort that went into incorporating Michael's new approaches have clearly been worth it.

One other thing that has changed in the past decade is the realization of how much our understanding of biology depends on our knowledge of development. If "nothing in biology makes sense except in the light of evolution," we now find

that "nothing in morphological evolution makes sense without knowledge of development." Changes in adult anatomy and physiology are predicated on changes in morphogenesis and differentiation during development.

This is also true of the history of biology, where developmental biology can be seen to play the unique role of "the stem cell of biological disciplines," constantly regenerating its own identity while simultaneously producing lineages that can differentiate in new directions. As Fred Churchill noted, *cell biology* "derived from descriptive embryology." The founders of cell biology were each trying to explain development, and their new conception of the cell helped them do it. The original theories of *evolution* concerned themselves with how new variants arose from the altered development of ancestors. Charles Darwin's friend and champion Thomas Huxley, expanded on this idea, which would eventually flourish into the field of *evolutionary developmental biology*.

Also during this Victorian age, a variant of developmental biology grew to become the field of *immunology*. Elie Metchnikoff (who showed the pole cells of flies to be germ cell precursors and who studied gastrulation throughout the animal kingdom) proposed a new cell theory of immunology in his attempt to find universal characters of the embryonic and larval mesoderm. Similarly, but with more anguish, *genetics* directly descended from a generation of embryologists who dealt with whether the nucleus or cytoplasm contained the determinants of embryonic development. Before his association with *Drosophila*, Thomas Hunt Morgan was a well-known embryologist who worked on sea urchin embryos, wrote a textbook on frog development, and was an authority on regeneration. Many of the first geneticists were originally embryologists, and it was only in the 1920s that Morgan formally separated the two fields. And *regeneration* is still intimately linked with development, for regeneration often is a recapitulation of embryonic processes. Ross Granville Harrison and Santiago Ramon y Cajal founded the science of *neurobiology* by showing how the brain and axons develop. To this day, neurology requires an understanding of the developmental origins of the central and peripheral nervous systems.

Several medical disciplines descend from embryology. *Teratology* (the study of congenital anomalies) has always studied altered or disrupted development, but other medical disciplines also trace back to embryology. *Cancer biology*—oncology—derives from developmental biology, as cancers have long been perceived and studied as a cell's reversion to an embryonic state. Although this view was at one time eclipsed by a strictly genetic view of cancer, today it is being revived and revised by the discoveries of cancer stem cells, paracrine factor regulation of tumor initiation, and embryonic modes of cell migration used by tumor cells. Medical disciplines such as cardiology and diabetes research are being invigorated by new developmental perspectives. And the new fields of *endocrine disruption* and the *developmental origin of health and disease*, looking at how environmental factors experienced during pregnancy can alter adult phenotypes, have emerged from developmental biology with their own paradigms and rules of evidence.

The developmental biology stem cell produces new disciplines even as it keeps its own identity. The field of *stem cell biology* is directly linked to its parent discipline, and new studies (many of them documented in this text) show how directing stem cells to differentiate in particular ways demands knowledge of their normal development.

Developmental biology interacts with other disciplines to induce new ways of thinking. Ecological developmental biology, for instance, looks at the interactions between developing organisms and their abiotic and biotic environments. Even the field of paleontology has been revolutionized by developmental perspectives that allow new and often surprising phylogenies to be constructed.

In short, this is an exciting time for this textbook to promote an interactive way of perceiving and studying the natural world. Pascal wrote that science is like a balloon expanding into the unknown. The more that we know, the greater the area in contact with the unknown. Developmental biology is a discipline where the unknown contains important questions yet to be answered, with new techniques and ideas for those ready to try.

Acknowledgments

It is becoming increasingly difficult to distinguish between an author, a curator, and a "nexus" in a node-link diagram. This book is a developing and symbiotic organism whose acknowledgments must either be confined to an inner layer or else expand throughout the world. First and foremost, I sincerely acknowledge that without Michael Barresi's enthusiasm, expertise, and passion for this project, this edition of the book would not exist.

The Sinauer Associates team, headed by Andy Sinauer and Rachel Meyers, has been remarkable. I have been incredibly privileged over the years to work with Sinauer Associates. I am also lucky to have had my words, sentences, and paragraphs, rearranged, reordered, and realigned by Carol Wigg, who has worked with me on all eleven editions to communicate the wonder of developmental biology in prose that is as clear, accessible, and enjoyable for students as we can possibly make it.

This is a beautiful book, and I can say that because it is not my doing. It is due to talent of Chris Small and his production staff; to Jefferson Johnson and his artistic mastery of Adobe InDesign; to the expertise of the artists at Dragonfly Media Graphics; and to photo editor extraordinaire David McIntyre, who manages to find incredible photographs to complement the many wonderful images my colleagues have so generously supplied for each edition.

I have been blessed with remarkable students who have never been shy about asking me questions. Even today they continue to send me "Did you see this?" emails that make sure I'm keeping current. I also thank all those people who continue to send me emails of encouragement or who come up to me at meetings to pass on good words about the book and provide me with even more information. This book is, and always has been, a community endeavor.

My wife, Anne Raunio, has put up with my textbook writing for most of our married life, and I know she'll be glad that this edition is finished. Indeed, just as this book goes to press our lives have shifted greatly with our move away from Swarthmore. I would certainly be remiss if I didn't acknowledge the many years of support I have enjoyed at Swarthmore College, a wonderful academic institution that deems textbook writing a service to the scientific community and that encourages interdisciplinary ventures.

—S.F.G.

From Michael Barresi

A NEUROSCIENTIST, AN ECO-EVO-DEVO BIOLOGIST, AND A DEVELOPMENTAL BIOLOGIST WALK INTO A POOL. Yes, it actually happened, on a scorching hot summer day in Cancun, Mexico! It was at the first Pan American Society for Developmental Biology Conference when Scott Gilbert mentioned to Kathryn Tosney and me that he was considering a co-author for the upcoming Eleventh Edition of *Developmental Biology*. While I waded in the water next to two of my heroes, Scott asked whether I might be interested in such an opportunity.

A combination of shock, excitement, and fear set in, pretty much in that order. *Shock*, because I was in wonderment of how I could be considered; after all, I had neither published a dozen papers a year nor had the historical perspective and cultural scope that Scott has woven so intricately and uniquely through each edition. *Excitement,* because this textbook has had such a great impact on my life. The chance to be part of a book that has been with me throughout my entire science education would be a true honor. Then *fear* set in because, as it does to me, this book means so much to so many in this field. The undertaking required to maintain the standard that Scott Gilbert has set for this work was daunting. However, if there is one thing I have learned in 11 years as a college professor, it is that fear can be the most significant barrier to innovative teaching and learning.

I agreed to be Scott's co-author because it presented an opportunity to influence how this subject is taught around the world. My enthusiasm for all aspects of the book is limitless, and I am passionately committed to improving the learning experience for all students. There is certainly no replacing Scott Gilbert, and I do not pretend to be Scott's equivalent. What I can offer to this and future editions of *Developmental Biology* is a complementary approach that builds upon Scott's accuracy and style with increased creativity and an overarching philosophy of student empowerment to learn about developmental biology.

The textbook and the classroom have something in common. Neither can survive this digital age as a mere vessel for information: pages of dense content paired with even denser lectures are not effective methods for "deep" learning. There is overwhelming evidence that true active learning pedagogies provide the most effective gains in conceptual understanding, longer retention of material, better problem-solving abilities, and greater persistence in STEM majors, particularly for underprepared students (Waldrop 2015; Freeman et al. 2014; Michelene et al. 2014). I want my students and yours to learn the core concepts in developmental biology not by simply memorizing the text or stressfully scribing bullets off of a PowerPoint, but by *experiencing* how these concepts can explain known and unknown phenomena of development. How can a textbook adapt to (1) support teachers in implementing effective active learning approaches, and (2) encourage students to become active learners?

Carrying out effective active learning exercises in class that target concept acquisition and problem solving skill development is challenging. Potential challenges include a lack of activities to offer students and a lack of training on behalf of the instructor to administer those exercises, a shortage of available class time (real or perceived), student reluctance to participate in novel and challenging activities, uneven preparedness by students, and a whole range of associated fears.

We have transformed the Eleventh Edition of *Developmental Biology* to support a movement in pedagogy toward an active experience for both the professor and student. For many of the chapters, Scott Gilbert and I have written and produced "Dev Tutorials," short (10–20 minutes) video recordings of us explaining some of the basic principles of development. These professionally produced videos are designed to deliver some basic amount of content outside of class, thus providing instructors with a mechanism to conduct a "flipped" classroom (see Seery 2015).

To satisfy the in-class half of the "flipped" classroom, we wrote a set of case study problems that accompany the "Dev Tutorials" to encourage team-based learning approaches. Prior to conducting a case study activity, consider asking students to read the "Punchline" for a specific chapter as well as watching the related "Dev Tutorial." Completing this won't take students very long, so instructors can expect that each student will walk into class with a baseline of content exposure sufficient to *actively* engage in solving the case study. We intend to add more "Dev Tutorials" and "Case Studies" in the future, as user interest demands. We are excited to see how "Dev Tutorials" and case study problems can be tailored to meet the learning objectives of your own courses, and I, in particular, welcome the chance to work with faculty to help support their implementation of these new active learning resources.

Traditionally, the role of a textbook has been to introduce students to the core concepts of a given field; however, I don't feel this should be its only role. Textbooks can take advantage of the fact that, usually, the student is reading about the subject for the first time. This is the moment to capture a student's inquisitive spirit, build their confidence in discussing and asking questions about the subject, and fuel their future learning through a determined ownership of their place in the field. Gaining a sense of identity in a particular field of science often begins with the ability to engage in a dialogue. Unfortunately, for a student learning "the facts" for the first time, one of the most difficult barriers is being able to articulate the questions that would open up a substantive conversation.

Several unique mechanisms in the Eleventh Edition are intended to empower students to engage actively with the field of developmental biology. The "Developing Questions" found throughout each chapter function as suggested extensions and potential areas of future research on the topics being covered, and indirectly provide a model for the type of thinking and questions that developmental biologists might ask. These questions would be a huge success if students repeated them in class as a sort of ice-breaker to begin or further the discussion, or used them as entry points for supplemental literature research on their own. Most of these questions do not have definitive answers. Sorry, but they are designed to spur interaction in the classroom and engage students with the actual research. The potential of the thrill of discovery to motivate student interest cannot be underestimated. And students know the difference between quiz questions and life questions. To that end, each chapter ends with a "Next Step Investigation"; these play a similar role to the "Developing Questions," except they attempt to present a broader view of the directions the field may be moving in. The hope is that students can use "Next Step Investigations" as logical entry points for their own research.

One other objective for the Eleventh Edition has been to introduce the actual voices of the biologists working today. "Scientists Speak" is a new resource linked throughout the textbook to provide students (and faculty) with direct access to recorded conversations with leading developmental biologists. Many of these discussions took place between the lead investigators from current and seminal papers and my own students at Smith College through web conferencing technology. For students, the unique benefit of this type of resource is a highly approachable dialogue with the scientists combined with a fantastic array of questions asked by their peers—often the only individuals students really trust.

I sincerely hope these many new resources help to increase student engagement, improve their confidence to communicate, and truly invite everyone to become a significant participant in this most amazing science of development.

Acknowledgments

I wish to express my special and sincere thanks to Mary Tyler, who played a pivotal role as a content editor for my chapters. Mary has held a great love for this textbook over the years, and her perspectives helped me achieve a perfect balance between the past and present in this new edition. Thank you, Mary, for all of your support and focused, substantive input.

The field of developmental biology is ever-expanding and the pace of research seems like it is increasing exponentially. This comprehensive edition was only possible with the keen oversight of the expert reviewers listed on the following page. Thanks to Johannah Walkowicz for her unique balance of persistence and kindness in organizing all of the reviewers. I extend a special acknowledgmentf to Willy Lensch and Bill Anderson, who spent significant time with me discussing the field of stem cells, which directly influenced the organization of the new stem cell chapter.

I have been continually amazed by the stellar team at Sinauer Associates, Publishers. I have been humbled by Andy Sinauer's complete acceptance of me into this family. His open-minded consideration of all of my ideas was a critical factor in my acceptance to co-author this great book; thank you Andy for your support, and for compiling the most amazing staff! First Azelie Aquadro Fortier and then Rachel Meyers oversaw the entire production of this edition, and both provided this new co-author nothing but genuine encouragement and support at all times. Carol Wigg, Sydney Carroll, and Laura Green worked together to provide the precise editorial eyes needed, especially for this tired, father-of-four, first-time author. Your determination and equally long hours on this project produced a new edition that I know I can only be proud of because of your contributions.

I sincerely appreciate the vast amount of energy and time Sinauer's art director Chris Small and the entire group at Dragonfly Media Graphics took to produce such a beautiful art program. They also had to deal with me, an overprotective visual artist who was likely too critical to changes to his original drawings! Thanks for your patience. I'd also like to thank Chris again, as well as Joanne Delphia and Jefferson Johnson, for their excellent design and layout of the book. David McIntyre, thank you for your help in researching and obtaining the many new photographs.

A new book can only reach the hands of the students with the help of strategic marketing, and Dean Scudder, Marie Scavotto, and Susan McGlew have been remarkable in highlighting all of the new features. I thank you for always managing to present this new author in the best light. Jason Dirks and all of the people working in Sinauer's Media and Supplements department deserve a special thanks for designing an appealing website and brainstorming with me about the best ways to present all of our new interactive features.

The support of Smith College cannot go unrecognized. Smith has allowed me to produce and disseminate my "Web Conferences," "Developmental Documentaries," and the "Dev Tutorials" used in this text. The commitment and talent of Kate Lee and the overall support by Smith's education technology services department have also made the production of these features possible. I would be remiss if I did not thank all of the scientists who over the years have volunteered their time to speak with my students about their research. Hopefully your shared insights will now reach many more students.

To my students at Smith College, both in my courses and in my research lab, I thank you for being my collaborators and the best teachers I have ever had. Your enthusiasm, hard work, and crazy ideas make all that I do worth it.

There are many things we do in our lives that could not be possible without the support of family. However, in my experience, I have never had to rely on my family quite as much as was required for this endeavor. True sacrifices were made by all in my family to meet the demands of this work. In my book, you are all my co-authors! I thank you for your unconditional love and support.

—M.J.F.B.

Reviewers of the Eleventh Edition

William Anderson, *Harvard University*
David Angelini, *Colby College*
Robert Angerer, *University of Rochester* and *NIH*
John Belote, *Syracuse University*
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Media and Supplements

to accompany **Developmental Biology**, *Eleventh Edition*

For the Student

Companion Website

devbio.com

Significantly enhanced for the Eleventh 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.
- **Web Topics**: 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 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 introduced in the textbook.
- **Bibliography**: Full citations are provided for all of the literature cited in the textbook (most linked to their PubMed citations).

DevBio Laboratory: Vade Mecum³: An Interactive Guide to Developmental Biology

labs.devbio.com

Mary S. Tyler and Ronald N. Kozlowski

Included with each new copy of the textbook, *Vade Mecum*³ is an interactive website that helps students understand the organisms discussed in the course, and prepare them for the lab. The site includes videos of developmental processes and laboratory techniques, and has chapters on the following organisms: slime mold (*Dictyostelium discoideum*), planarian, sea urchin, fruit fly (*Drosophila*), chick, and amphibian. (Also available for purchase separately.)

Developmental Biology: A Guide for Experimental Study, Third Edition

(Included in *DevBio Laboratory: Vade Mecum*³)

Mary S. Tyler

This lab manual teaches students to work as independent investigators on problems in development and provides extensive background information and instructions for each experiment. It emphasizes the study of living material, intermixing developmental anatomy in an enjoyable balance, and allows students to make choices in their work.

For the Instructor

(Available to qualified adopters)

Instructor's Resource Library

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

- **Case Studies in Dev Bio**: This new collection of case study problems accompanies the Dev Tutorials and 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**: Answers, references, and recommendations for further reading are provided so that you and your students can explore the Developing Questions that are posed throughout each chapter.
- **Textbook Figures & Tables**: All of the textbook's figures, photos, and tables are provided both in JPEG (high- and low-resolution) and PowerPoint formats. All images have been optimized for excellent legibility when projected in the classroom.
- **Video Collection**: Includes video segments depicting a wide range of developmental processes, plus segments from *DevBio Laboratory: Vade Mecum*³, and *Differential Experessions*². For each video collection, an instructor's guide is provided.
- ***Vade Mecum*³ PowerPoints**: Chick serial sections and whole mounts, provided in both labeled and unlabeled versions, for use in creating quizzes, exams, or in-class exercises.
- ***Developmental Biology: A Guide for Experimental Study*, Third Edition** (by Mary S. Tyler): The complete lab manual, in PDF format.

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Developmental Biology 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

Making New Bodies

Mechanisms of Developmental Organization

What stays the same when a tadpole becomes a frog, and what changes?

BETWEEN FERTILIZATION AND BIRTH, the developing organism is known as an embryo. 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. 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. 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 nearly all cases, the development of a multicellular organism begins with a single cell—the fertilized egg, or **zygote**, which 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. 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. Some animals can regenerate severed parts, and many species undergo metamorphosis (such as the transformation of a tadpole into a frog, or a caterpillar into a butterfly).

The Punchline

Animal development is characterized by the differentiation of the fertilized egg into the many cell types of the body and by the construction of functionally integrated organs. Development is the route via which an organism goes from genotype to phenotype, and it can be studied at any level of organization, from molecules to ecosystems. The processes of development include fertilization, cleavage, gastrulation, organogenesis, metamorphosis, regeneration, and senescence. These processes are among the greatest sources of questions in science, questions such as: How do the various cell types—blood cells, neurons, pancreas cells, etc.—form, and how do they become different from one another? How do the cells become organized into functional organs? How do the organs know their correct size? How do organisms make cells that can reproduce? How can organisms regenerate tissues and missing parts? How can the organism integrate cues from the environment to develop properly? And how can the pathways of development change to produce new types of organisms?

Therefore, in recent years it has become customary to speak of **developmental biology** as the discipline that studies embryonic and other developmental processes.

"How Are You?" The Questions of Developmental Biology

Aristotle, the first known embryologist, said that wonder was the source of knowledge, and animal development, as Aristotle knew well, is a remarkable source of wonder. This development, this formation of an orderly body from relatively homogeneous material, provokes profound and fundamental questions that *Homo sapiens* have asked since the dawn of self-awareness: How does the body form with its head always above its shoulders? How come the heart is 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? 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!*

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 fertilized egg 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:

- **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?[1]
- **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. Our

[1] 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. A particular cell may play many roles during development, going through stages that are no longer seen in adulthood. In addition, the role of some cell types is to activate specific genes in neighboring cells, and once this function is accomplished, the activating cell type dies. The primary notochord cells, for example, are not even listed in medical histology texts. Once this task is done, most of them undergo programmed cell death so as not to disturb further neural development. Because such a cell type is not seen in the adult, it and its importance are known mainly by developmental biologists.

fingers are always at the tips of our hands, never in the middle; our eyes are always in our heads, not in our toes or gut. This creation of ordered form is called **morphogenesis**, and it involves coordinating cell growth, cell migration, and cell death.

- **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?
- **The question of reproduction** The sperm and egg are highly specialized cells, and only they 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?
- **The question of regeneration** Some organisms can regenerate every part of their bodies. Some salamanders regenerate their eyes and their legs, while 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?
- **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 egg. 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?
- **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. The study of development has become essential for understanding all other areas of biology. In turn, the many advances of molecular biology, along with new techniques of cell imaging, have finally made these questions answerable. This is exciting; for, as the Nobel-prize winning developmental biologist Hans Spemann stated in 1927, "We stand in the presence of riddles, but not without the hope of solving them. And riddles with the hope of solution—what more can a scientist desire?"

So, we come bearing questions. They are questions bequeathed to us by earlier generations of biologists, philosophers, and parents. They are questions with their own histories, questions discussed on an anatomical level by people such as Aristotle, William Harvey, St. Albertus Magnus, and Charles Darwin. More recently, these questions have been addressed on the cellular and molecular levels by men and women throughout the world, each of whom brings to the laboratory his or her own perspectives and training. For there is no one way to become a developmental biologist, and the field has benefitted by having researchers trained in cell biology, genetics, biochemistry, immunology, and even anthropology, engineering, physics, and art.

The Cycle of Life

For animals, fungi, and plants, the sole way of getting from egg to adult is by developing an embryo. The embryo is where genotype is translated into phenotype, where inherited genes are expressed to form the adult. The developmental biologist usually finds the transient stages leading up to the adult to be the most interesting. Developmental biology studies the building of organisms. It is a science of becoming, a science of process.

One of the major triumphs of descriptive embryology was the idea of a generalizable animal life cycle. Modern developmental biology investigates the temporal changes of gene expression and anatomical organization along this life cycle. Each animal, whether earthworm or eagle, termite or beagle, passes through similar stages of development: fertilization, cleavage, gastrulation, organogenesis, 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 extremely rapid 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**.
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 many 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 a called a **larva**, and it may look significantly different from the adult. In many 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 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 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.

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."**

WEB TOPIC 1.1 **WHEN DOES A HUMAN BECOME A PERSON?** Scientists have proposed different answers to this question. Fertilization, gastrulation, the first signs of brain function, and the time around birth—each of these stages has its supporters as the starting point of human personhood.

VADE MECUM

As seen in the segment on amphibians, frogs display some of the most dramatic of vertebrate life cycles.

An Example: A Frog's Life

All animal life cycles are modifications of the generalized one described above. Here we will present a concrete example, the development of the leopard frog *Rana pipiens* (**FIGURE 1.1**).

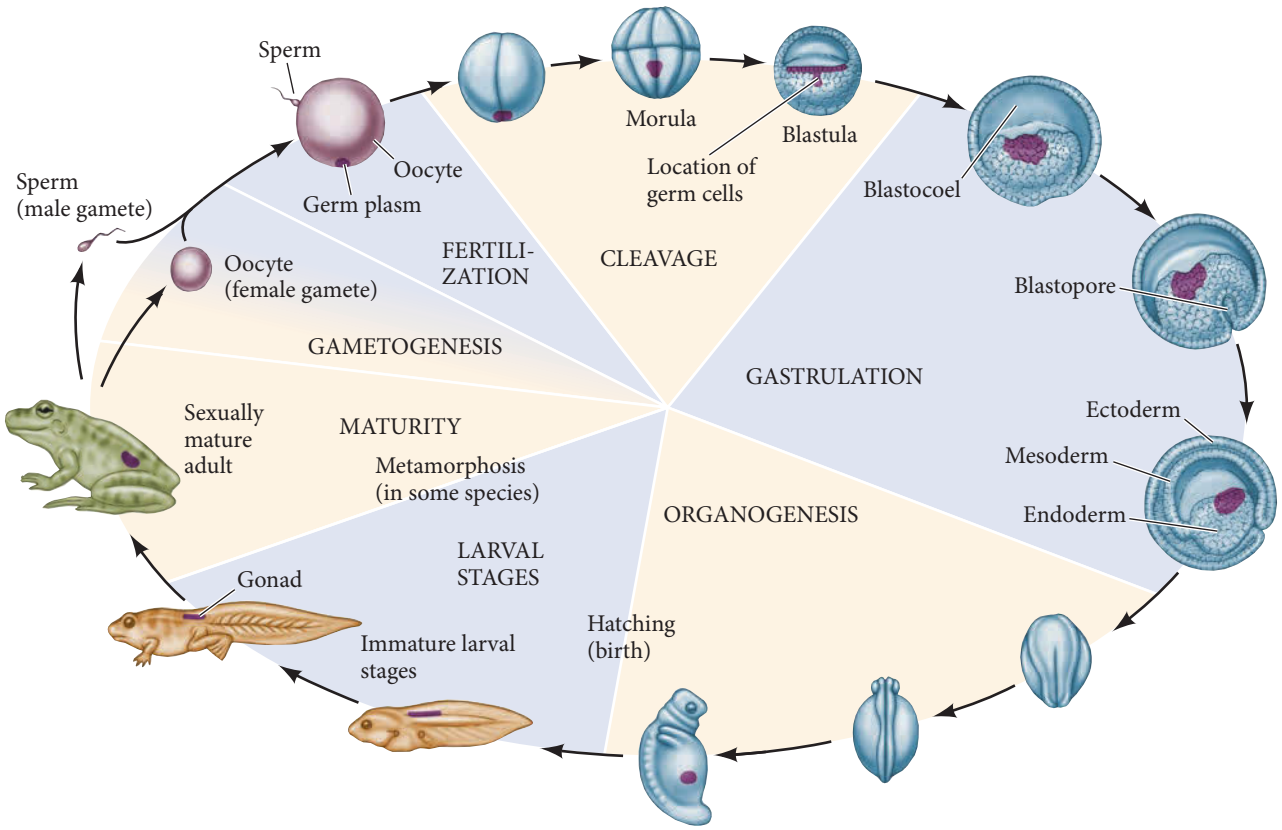

FIGURE 1.1 Developmental history of the leopard frog, *Rana pipiens*. The stages from fertilization through hatching (birth) are known collectively as embryogenesis. The region set aside for producing germ cells is shown in purple. Gametogenesis, which is completed in the sexually mature adult, begins 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 1.2A**). The male frog grabs the female's back and fertilizes the eggs as the female releases them (**FIGURE 1.2B**). 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 involved 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 1.2C,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 1.2E**). 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 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 a rod of cells called the **notochord**.[2] 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 1.2F**). 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 1.2G**). The neurons make connections to the muscles and to other

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

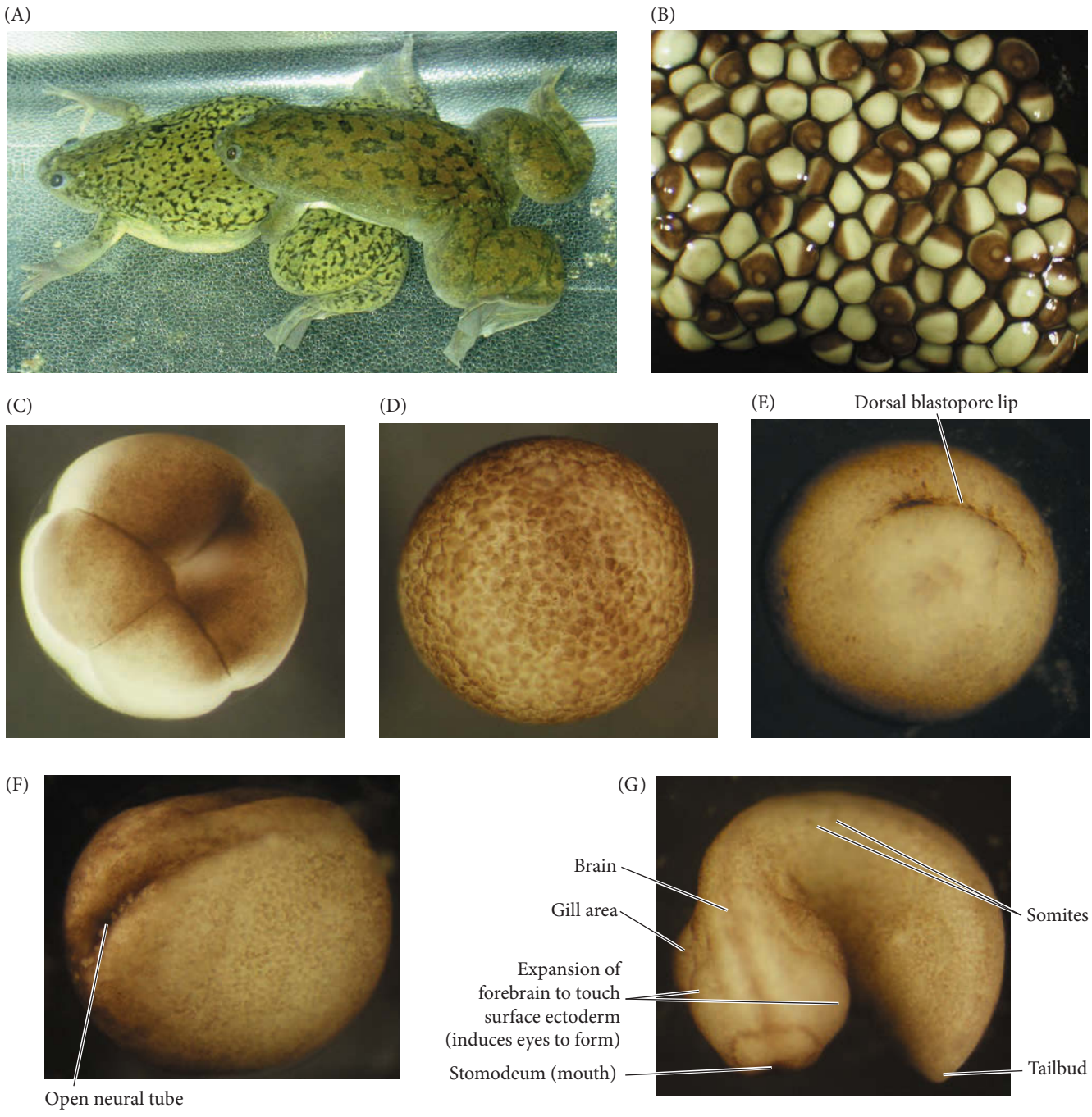

FIGURE 1.2 Early development of the frog *Xenopus laevis*. (A) Frogs mate by amplexus, the male grasping the female around the belly and fertilizing the eggs as they are released. (B) A newly laid clutch of eggs. The cytoplasm has rotated such that the darker pigment is where the nucleus resides. (C) 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. (Courtesy of Michael Danilchik and Kimberly Ray.)

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 and very obvious (**FIGURE 1.3**). 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 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 egg) 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**. 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.

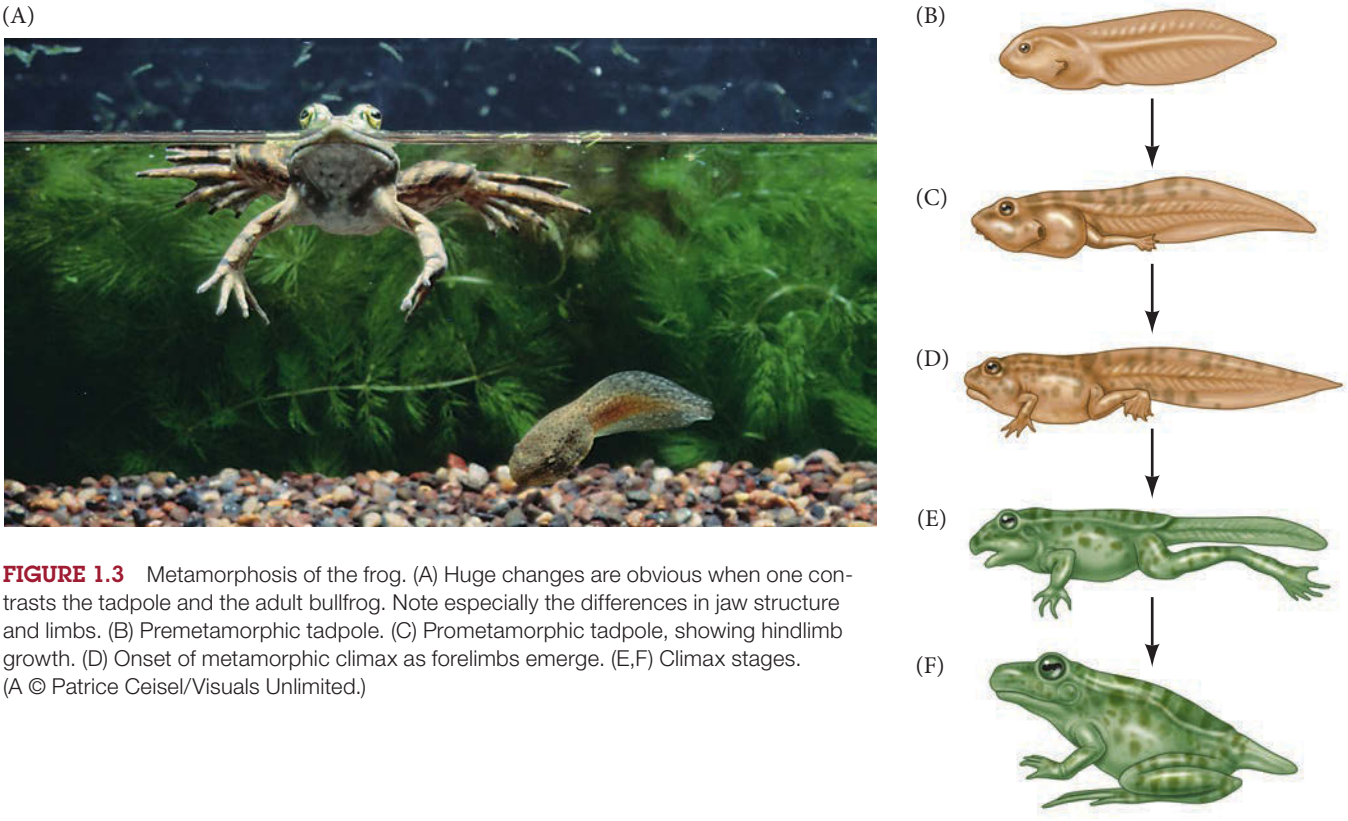

FIGURE 1.3 Metamorphosis of the frog. (A) Huge changes are obvious when one contrasts the tadpole and the adult bullfrog. Note especially the differences in jaw structure and limbs. (B) Premetamorphic tadpole. (C) Prometamorphic tadpole, showing hindlimb growth. (D) Onset of metamorphic climax as forelimbs emerge. (E,F) Climax stages. (A © Patrice Ceisel/Visuals Unlimited.)

Comparative Embryology

The fertilized egg has no heart. Where does the heart come from? Does it form the same way in both insects and vertebrates? How is heart development in these two groups similar and how is it different? How do the tissues that form a bird's wing relate to the tissues that form a fish fin or a human hand? Many of the questions in developmental biology are of this type, and they stem from the field's embryological heritage. The first known study of comparative developmental anatomy was undertaken by Aristotle. In *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 the spontaneous generation of animals from mud or excrement.[3] 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 1.4**). Marcello Malpighi published the first microscopic account of chick development in 1672. Here, for the first time, the neural groove (precursor of the neural tube), the muscle-forming somites, and the first circulation of the arteries and veins—to and from the yolk—were identified.

Epigenesis and preformationism

With Malpighi began one of the great debates in embryology: the controversy over whether the organs of the embryo are formed de novo ("from scratch") at each generation, or whether the organs are already present, in miniature form, within the egg or sperm. The first view, **epigenesis**, was supported by Aristotle and Harvey. The second view, **preformationism**, was reinvigorated with Malpighi's support. Malpighi showed that the unincubated[4] chick egg already had a great deal of structure, and this observation provided him with reasons to question epigenesis and advocate the preformationist view, according to which all the organs of the adult were prefigured in miniature within the sperm or (more usually) the egg. Organisms were not seen to be "constructed" but rather "unrolled" or "unfurled."

The preformationist view had the backing of eighteenth-century science, religion, and philosophy (Gould 1977; Roe 1981; Churchill 1991; Pinto-Correia 1997). First, if all organs were prefigured, embryonic development merely required the growth of existing structures, not the formation of new ones. No extra mysterious force was needed for

[3] 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.

[4] As pointed out by Maître-Jan in 1722, the eggs Malpighi examined may technically be called "unincubated," but as they were left sitting in the Bolognese sun in August, they were not unheated. Such eggs would be expected to have developed into chicks.