

Developmental Biology

Eleventh
Edition

Gilbert ■ Barresi



INCLUDED WITH THIS BOOK

DevBio Laboratory: *Vade Mecum*³

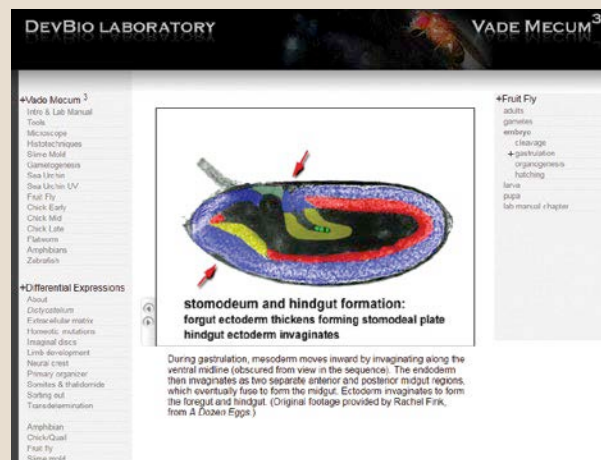
An Interactive Guide to Developmental Biology

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 Mecum*³ 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:

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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.

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



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 pri-

Jack Schultz (originally in Morgan's laboratory) and others, a burgeoning supply of data on the genetics of *Drosophila* to its day was a difficult organism on which to study embryology. Fly embryos are small 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.

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.

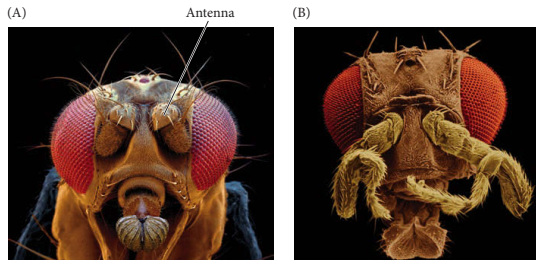


FIGURE 9.26 (A) Head of a wild-type fruit fly. (B) Head of a fly containing the *Antennapedia* mutation that converts antennae into legs. (A © Eye of Science/ Science Source; B © Science VU/Dr. F. Rudolph Turner/Visuals Unlimited, Inc.)

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.

Generating the Dorsal-Ventral Axis

Dorsal-ventral patterning in the oocyte

As oocyte volume increases, the oocyte nucleus is pushed by the growth to an anterior dorsal position (Zhao et al. 2012). Here the *gurken* messenger RNA, which initiates the anterior-posterior axis, initiates the dorsal-ventral axis. The *gurken* mRNA is localized in a crescent

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?

Developing Questions

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

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 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**

- 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.
 - 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.
 - 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* in which genes are transcribed, and often regulate the expression of the next set of genes.
 - Boundaries* of gene expression are established through 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

Snapshot Summary

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

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.

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.

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 (Hengge 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.

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

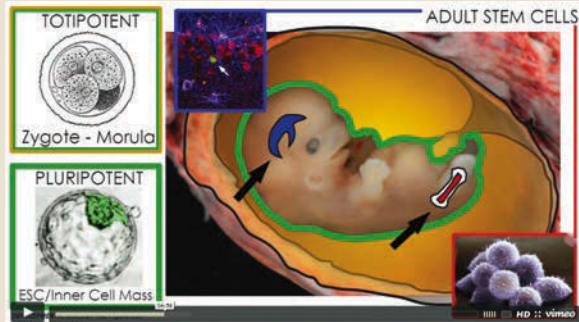
A cell is a stem cell if it can divide and in doing so produce a replica of itself (a process called **self-renewal**) as well as a daughter cell that can undergo further development. Stem cells are often referred to as undifferentiated due to this maintenance of proliferative properties¹. Upon division, 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

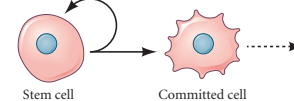
¹There are many different stem cells and so their status as "undifferentiated" really only pertains to the retained ability to divide, but they are in fact a defined cell type.

Dev Tutorial

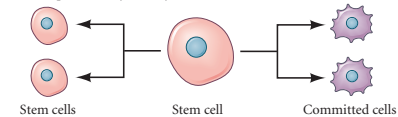
Providing additional ways for you to explore topics, these video tutorials, presented by the book's authors, reinforce key concepts from the text.



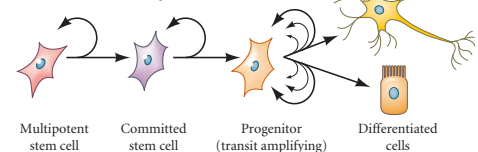
(A) Single-cell asymmetry



(B) Population asymmetry



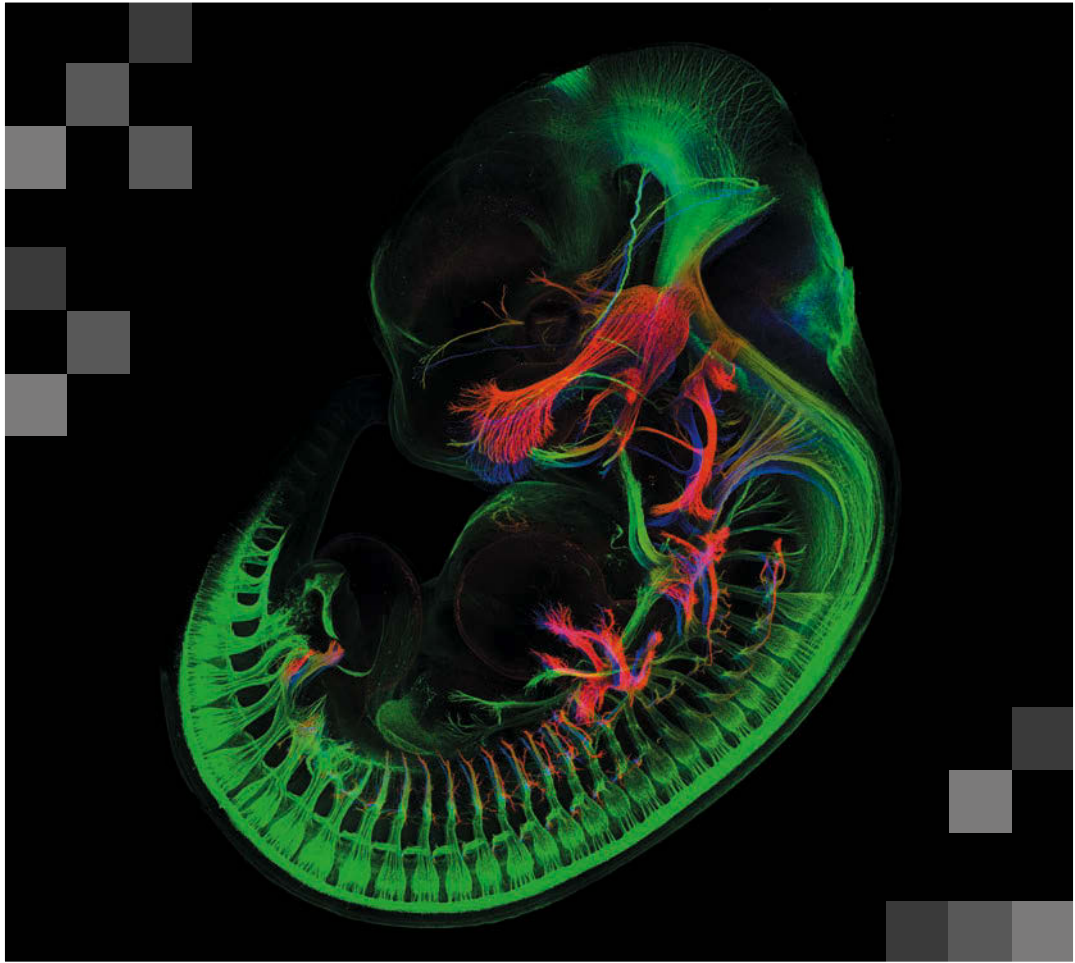
(C) Adult stem cell lineage



stem cell concept. (A) The fundamental concept is that it can make a copy of itself while also producing cells committed to a specific fate. This is called asymmetric division. A population of stem cells is maintained through population asymmetry. A stem cell is shown to have the ability to produce either two stem cells (thus decreasing the size of the stem cell pool by 1) or to produce two differentiated cells (thus increasing the size of the differentiated cell pool). (B) In many organisms, stem cells are maintained through population asymmetry. A population of stem cells is maintained through population asymmetry. A stem cell is shown to have the ability to produce either two stem cells (thus decreasing the size of the stem cell pool by 1) or to produce two differentiated cells (thus increasing the size of the differentiated cell pool). (C) In many organisms, stem cells are maintained through population asymmetry. A population of stem cells is maintained through population asymmetry. A stem cell is shown to have the ability to produce either two stem cells (thus decreasing the size of the stem cell pool by 1) or to produce two differentiated cells (thus increasing the size of the differentiated cell pool).

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■ Eleventh Edition



Scott F. Gilbert

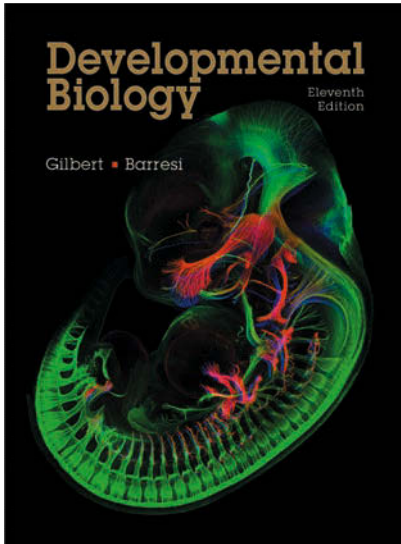
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The Cover

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.

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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

I Patterns and Processes of Becoming: A Framework for Understanding Animal Development

- CHAPTER 1 ■ **Making New Bodies:** Mechanisms of Developmental Organization 1
- CHAPTER 2 ■ **Specifying Identity:** Mechanisms of Developmental Patterning 29
- CHAPTER 3 ■ **Differential Gene Expression:** Mechanisms of Cell Differentiation 45
- CHAPTER 4 ■ **Cell-to-Cell Communication:** Mechanisms of Morphogenesis 95
- CHAPTER 5 ■ **Stem Cells:** Their Potential and Their Niches 143

II Gametogenesis and Fertilization: The Circle of Sex

- CHAPTER 6 ■ **Sex Determination and Gametogenesis** 181
- CHAPTER 7 ■ **Fertilization:** Beginning a New Organism 217

III Early Development: Cleavage, Gastrulation, and Axis Formation

- CHAPTER 8 ■ **Rapid Specification in Snails and Nematodes** 251
- CHAPTER 9 ■ **The Genetics of Axis Specification in *Drosophila*** 277
- CHAPTER 10 ■ **Sea Urchins and Tunicates:** Deuterostome Invertebrates 311
- CHAPTER 11 ■ **Amphibians and Fish** 333
- CHAPTER 12 ■ **Birds and Mammals** 379

IV Building with Ectoderm: The Vertebrate Nervous System and Epidermis

- CHAPTER 13 ■ **Neural Tube Formation and Patterning** 413
- CHAPTER 14 ■ **Brain Growth** 439
- CHAPTER 15 ■ **Neural Crest Cells and Axonal Specificity** 463
- CHAPTER 16 ■ **Ectodermal Placodes and the Epidermis** 517

V Building with Mesoderm and Endoderm: Organogenesis

- CHAPTER 17 ■ **Paraxial Mesoderm:** The Somites and Their Derivatives 539
- CHAPTER 18 ■ **Intermediate and Lateral Plate Mesoderm:** Heart, Blood, and Kidneys 581
- CHAPTER 19 ■ **Development of the Tetrapod Limb** 613
- CHAPTER 20 ■ **The Endoderm:** Tubes and Organs for Digestion and Respiration 653

VI Postembryonic Development

- CHAPTER 21 ■ **Metamorphosis:** The Hormonal Reactivation of Development 671
- CHAPTER 22 ■ **Regeneration** 693
- CHAPTER 23 ■ **Aging and Senescence** 723

VII Development in Wider Contexts

- CHAPTER 24 ■ **Development in Health and Disease:** Birth Defects, Endocrine Disruptors, and Cancer 735
- CHAPTER 25 ■ **Development and the Environment:** Biotic, Abiotic, and Symbiotic Regulation of Development 763
- CHAPTER 26 ■ **Development and Evolution:** Developmental Mechanisms of Evolutionary Change 785

Contents

PART I ■ **Patterns and Processes of Becoming:** A Framework for Understanding Animal Development



CHAPTER 1 **Making New Bodies:** Mechanisms of Developmental Organization **1**

“How Are You?” The Questions of Developmental Biology 2

The Cycle of Life 4

An Example: A Frog’s Life 5

Gametogenesis and fertilization 6

Cleavage and gastrulation 6

Organogenesis 6

Metamorphosis and gametogenesis 8

Comparative Embryology 9

Epigenesis and preformationism 9

An Overview of Early Development 11

Patterns of cleavage 11

Gastrulation: “The most important time in your life” 14

Naming the parts: The primary germ layers and early organs 14

The four principles of Karl Ernst von Baer 15

Keeping Track of Moving Cells: Fate Maps and Cell Lineages 17

Fate maps 18

Direct observation of living embryos 19

Dye marking 19

Genetic labeling 20

Transgenic DNA chimeras 21

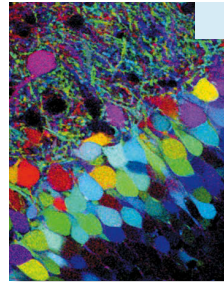
Evolutionary Embryology 23

Embryonic homologies 24

Medical Embryology and Teratology 26

Genetic malformations and syndromes 26

Disruptions and teratogens 26



CHAPTER 2 **Specifying Identity:** Mechanisms of Developmental Patterning **29**

Levels of Commitment 30

Cell differentiation 30

Commitment 30

Autonomous Specification 32

Cytoplasmic determinants and autonomous specification in the tunicate 32

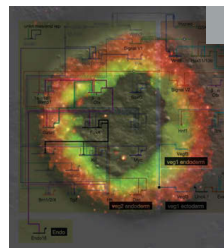
Conditional Specification 35

Cell position matters: Conditional specification in the sea urchin embryo 36

Syncytial Specification 38

Opposing axial gradients define position 39

A Rainbow of Cell Identities 41



CHAPTER 3 **Differential Gene Expression:** Mechanisms of Cell Differentiation **45**

Defining Differential Gene Expression 45

Quick Primer on the Central Dogma 46

Evidence for Genomic Equivalence 47

Modulating Access to Genes 50

Loosening and tightening chromatin: Histones as gatekeepers 50

Maintaining a memory of methylation 52

Anatomy of the Gene 52

Exons and introns 52

Cis regulatory elements: The on, off, and dimmer switches of a gene 55

Transcription factor function 61

The Gene Regulatory Network: Defining an Individual Cell 67

Mechanisms of Differential Gene Transcription 68

Differentiated proteins from high and low CpG-content promoters 68

DNA methylation, another key on/off switch of transcription 69

Differential RNA Processing 73

Creating families of proteins through differential mRNA splicing 73

Splicing enhancers and recognition factors 76

Control of Gene Expression at the Level of Translation 76

Differential mRNA longevity 77

Stored oocyte mRNAs: Selective inhibition of mRNA translation 78

Ribosomal selectivity: Selective activation of mRNA translation 80

microRNAs: Specific regulation of mRNA translation and transcription 80

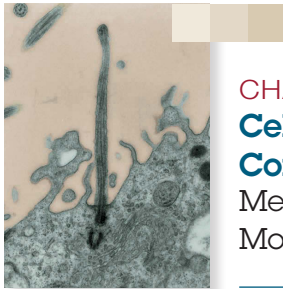
Control of RNA expression by cytoplasmic localization 83

Posttranslational Regulation of Gene Expression 84

The Basic Tools of Developmental Genetics 85

Characterizing gene expression 85

Testing Gene Function 88



CHAPTER 4 Cell-to-Cell Communication: Mechanisms of Morphogenesis 95

A Primer on Cell-to-Cell Communication 96

Adhesion and Sorting: Juxtacrine Signaling and the Physics of Morphogenesis 97

Differential cell affinity 97

The thermodynamic model of cell interactions 98

Cadherins and cell adhesion 100

The Extracellular Matrix as a Source of Developmental Signals 104

Integrins: Receptors for extracellular matrix molecules 106

The Epithelial-Mesenchymal Transition 107

Cell Signaling 108

Induction and competence 108

Reciprocal induction 110

Epithelial-mesenchymal interactions 112

The insect trachea: Combining inductive signals with cadherin regulation 114

Paracrine Factors: Inducer Molecules 115

Morphogen gradients 115

Signal transduction cascades: The response to inducers 116

Fibroblast growth factors and the RTK pathway 118

FGFs and the JAK-STAT pathway 120

The Hedgehog family 121

The Wnt family 125

The TGF- β superfamily 128

Other paracrine factors 130

The Cell Biology of Paracrine Signaling 130

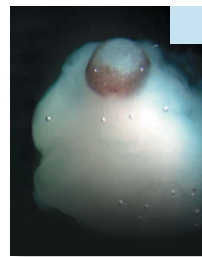
Focal membrane protrusions as signaling sources 134

Juxtacrine Signaling for Cell Identity 137

The Notch pathway: Juxtaposed ligands and receptors for pattern formation 137

Paracrine and juxtacrine signaling in coordination: Vulval induction in *C. elegans* 138

Hippo: An integrator of pathways 139



CHAPTER 5 Stem Cells: Their Potential and Their Niches 143

The Stem Cell Concept 144

Division and self-renewal 144

Potency defines a stem cell 145

Stem Cell Regulation 146

Pluripotent Cells in the Embryo 148

Cells of the inner cell mass 148

Mechanisms promoting pluripotency of ICM cells 148

Adult Stem Cell Niches 149

Stem cells fueling germ cell development in *Drosophila* 150

Adult Neural Stem Cell Niche of the V-SVZ 153

The neural stem cell niche of the V-SVZ 154

Maintaining the NSC pool with cell-to-cell interactions 155

Promoting differentiation in the V-SVZ niche 156

Environmental influences on the NSC niche 156

The Adult Intestinal Stem Cell Niche 158

Clonal renewal in the crypt 159

Regulatory mechanisms in the crypt 160

Stem Cells Fueling the Diverse Cell Lineages in Adult Blood 161

- The hematopoietic stem cell niche 161
- Regulatory mechanisms in the endosteal niche 163
- Regulatory mechanisms in the perivascular niche 163

The Mesenchymal Stem Cell: Supporting a Variety of Adult Tissues 164

- Regulation of MSC development 165

Other stem cells supporting adult tissue maintenance and regeneration 165

The Human Model System to Study Development and Disease 167

- Pluripotent stem cells in the lab 167
- Induced pluripotent stem cells 171
- Organoids: Studying human organogenesis in a culture dish 174

Stem Cells: Hope or Hype? 176**PART II ■ Gametogenesis and Fertilization: The Circle of Sex**

CHAPTER 6
**Sex Determination
and Gametogenesis 181**

Chromosomal Sex Determination 182**The Mammalian Pattern of Sex Determination 182****Primary Sex Determination in Mammals 184**

- The developing gonads 185
- Genetic mechanisms of primary sex determination:
Making decisions 187
- The ovary pathway: Wnt4 and R-spondin1 188
- The testis pathway: Sry and Sox9 189
- The right time and the right place 193

**Secondary Sex Determination in Mammals:
Hormonal Regulation of the Sexual
Phenotype 194**

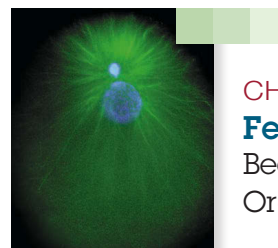
- The genetic analysis of secondary sex determination 195

Chromosomal Sex Determination in *Drosophila* 197

- The Sex-lethal gene 198
- Doublesex: The switch gene for sex determination 200

Environmental Sex Determination 201**Mammalian Gametogenesis 202**

- Meiosis: The intertwining of life cycles 205
- Gametogenesis in mammals: Spermatogenesis 207
- Gametogenesis in mammals: Oogenesis 211

Coda 212

CHAPTER 7
**Fertilization:
Beginning a New
Organism 217**

Structure of the Gametes 218

- Sperm 218
- The egg 220
- Recognition of egg and sperm 223

External Fertilization in Sea Urchins 223

- Sperm attraction: Action at a distance 224
- The acrosome reaction 225
- Recognition of the egg's extracellular coat 226
- Fusion of the egg and sperm cell membranes 228
- One egg, one sperm 228
- The fast block to polyspermy 230
- The slow block to polyspermy 230
- Calcium as the initiator of the cortical granule reaction 231

Activation of Egg Metabolism in Sea Urchins 233

- Release of intracellular calcium ions 234
- Effects of calcium release 236

Fusion of Genetic Material in Sea Urchins 238**Internal Fertilization in Mammals 239**

- Getting the gametes into the oviduct: Translocation and capacitation 240
- In the vicinity of the oocyte: Hyperactivation, thermotaxis, and chemotaxis 242
- The acrosome reaction and recognition at the zona pellucida 243
- Gamete fusion and the prevention of polyspermy 245
- Fusion of genetic material 246
- Activation of the mammalian egg 247

Coda 248

PART III ■ **Early Development: Cleavage, Gastrulation, and Axis Formation**



CHAPTER 8 **Rapid Specification in Snails and Nematodes 251**

Developmental Patterns among the Metazoa 252

- Basal phyla 252
- The triploblastic animals: Protostomes and deuterostomes 252

Early Development in Snails 254

Cleavage in Snail Embryos 255

- Maternal regulation of snail cleavage 256
- The snail fate map 258
- Cell specification and the polar lobe 259
- Altering evolution by altering cleavage patterns: An example from a bivalve mollusk 263

Gastrulation in Snails 265

The Nematode *C. Elegans* 265

Cleavage and Axis Formation in *C. elegans* 267

- Rotational cleavage of the egg 268
- Anterior-posterior axis formation 268
- Dorsal-ventral and right-left axis formation 269
- Control of blastomere identity 269

Gastrulation in *C. elegans* 272



CHAPTER 9 **The Genetics of Axis Specification in *Drosophila* 277**

Early *Drosophila* Development 278

- Fertilization 279
- Cleavage 279
- The mid-blastula transition 281
- Gastrulation 283

The Genetic Mechanisms Patterning the *Drosophila* Body 284

Segmentation and the Anterior-Posterior Body Plan 284

- Anterior-posterior polarity in the oocyte 285

Maternal gradients: Polarity regulation by oocyte cytoplasm 286

The anterior organizing center: The Bicoid and Hunchback gradients 292

The terminal gene group 293

Segmentation Genes 294

Segments and parasegments 294

The gap genes 295

The pair-rule genes 297

The segment polarity genes 298

The Homeotic Selector Genes 301

Generating the Dorsal-Ventral Axis 303

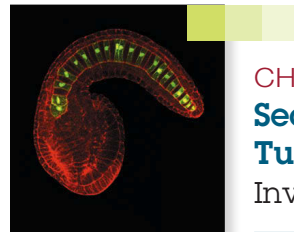
Dorsal-ventral patterning in the oocyte 303

Generating the dorsal-ventral axis within the embryo 305

Establishing a nuclear Dorsal gradient 305

Axes and Organ Primordia: The Cartesian Coordinate Model 306

Coda 307



CHAPTER 10 **Sea Urchins and Tunicates: Deuterostome Invertebrates 311**

Early Development in Sea Urchins 311

Early cleavage 312

Blastula formation 314

Fate maps and the determination of sea urchin blastomeres 314

Gene regulatory networks and skeletogenic mesenchyme specification 316

Specification of the vegetal cells 319

Sea Urchin Gastrulation 320

Ingression of the skeletogenic mesenchyme 320

Invagination of the archenteron 324

Early Development in Tunicates 326

Cleavage 327

The tunicate fate map 327

Autonomous and conditional specification of tunicate blastomeres 328



CHAPTER 11 Amphibians and Fish 333

Early Amphibian Development 333

Fertilization, Cortical Rotation, and Cleavage 334

- Unequal radial holoblastic cleavage 335
- The mid-blastula transition: Preparing for gastrulation 337

Amphibian Gastrulation 337

- Vegetal rotation and the invagination of the bottle cells 337
- Epiboly of the prospective ectoderm 342

Progressive Determination of the Amphibian Axes 343

- Specification of the germ layers 343
- The dorsal-ventral and anterior-posterior axes 344

The Work of Hans Spemann and Hilde Mangold 344

- Autonomous specification versus inductive interactions 344
- Primary embryonic induction 347

Molecular Mechanisms of Amphibian Axis Formation 348

- How does the organizer form? 349
- Functions of the organizer 355
- Induction of neural ectoderm and dorsal mesoderm: BMP inhibitors 355

Regional Specificity of Neural Induction along the Anterior-Posterior Axis 359

- The head inducer: Wnt antagonists 361
- Trunk patterning: Wnt signals and retinoic acid 363

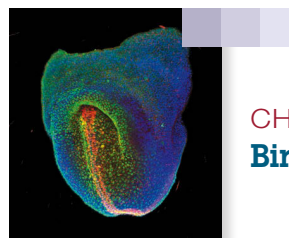
Specifying the Left-Right Axis 364

Early Zebrafish Development 365

Cleavage 368

Gastrulation and Formation of the Germ Layers 370

- Dorsal-ventral axis formation 374
- Anterior-posterior axis formation 376
- Left-right axis formation 376



CHAPTER 12 Birds and Mammals 379

Early Development in Birds 381

Avian Cleavage 381

Gastrulation of the Avian Embryo 382

- The hypoblast 382
- The primitive streak 382
- Molecular mechanisms of migration through the primitive streak 386
- Regression of the primitive streak and epiboly of the ectoderm 387

Axis Specification and the Avian “Organizer” 389

- The role of gravity and the PMZ 389
- Left-right axis formation 390

Early Development in Mammals 391

Cleavage 391

- The unique nature of mammalian cleavage 391
- Compaction 393
- Trophoblast or ICM? The first decision of the rest of your life 394
- Escape from the zona pellucida and implantation 395

Mammalian Gastrulation 396

- Modifications for development inside another organism 396

Mammalian Axis Formation 399

- The anterior-posterior axis: Two signaling centers 400
- Anterior-posterior patterning by FGF and RA gradients 401
- Anterior-posterior patterning: The Hox code hypothesis 402
- The left-right axis 404

Twins 406

Coda 408

PART IV ■ Building with Ectoderm:
The Vertebrate Nervous System and Epidermis



CHAPTER 13
Neural Tube Formation and Patterning 413

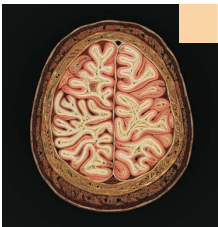
Transforming the Neural Plate into a Tube: The Birth of the Central Nervous System 415

- Primary neurulation 416
- Secondary neurulation 427

Patterning the Central Nervous System 428

- The anterior-posterior axis 428
- The dorsal-ventral axis 430
- Opposing morphogens 431
- Transcriptional cross-repression 434

All Axes Come Together 435



CHAPTER 14
Brain Growth 439

Neuroanatomy of the Developing Central Nervous System 440

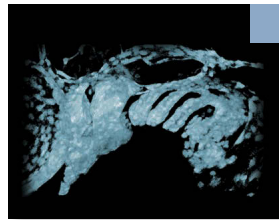
- The cells of the developing central nervous system 440
- Tissues of the developing central nervous system 443

Developmental Mechanisms Regulating Brain Growth 447

- Neural stem cell behaviors during division 447
- Neurogenesis: Building from the bottom up (or from the inside out) 448
- Glia as scaffold for the layering of the cerebellum and neocortex 450
- Signaling mechanisms regulating development of the neocortex 451

Development of the Human Brain 455

- Fetal neuronal growth rate after birth 455
- Hills raise the horizon for learning 456
- Genes for neuronal growth 459
- High transcriptional activity 460
- Teenage brains: Wired and unchained 460



CHAPTER 15
Neural Crest Cells and Axonal Specificity 463

The Neural Crest 463

- Regionalization of the Neural Crest 465**
- Neural Crest: Multipotent Stem Cells? 466**
- Specification of Neural Crest Cells 468**
- Neural Crest Cell Migration: Epithelial to Mesenchymal and Beyond 470**

- Delamination 471
- The driving force of contact inhibition 473
- Collective migration 473

Migration Pathways of Trunk Neural Crest Cells 474

- The ventral pathway 475
- The dorsolateral pathway 479

Cranial Neural Crest 481

The “Chase and Run” Model 483

Neural Crest-Derived Head Skeleton 484

- Coordination of face and brain growth 485

Cardiac Neural Crest 486

Establishing Axonal Pathways in the Nervous System 488

The Growth Cone: Driver and Engine of Axon Pathfinding 488

- “Plus tips” and actin-microtubule interactions 490
- Rho, Rho, Rho your actin filaments down the signaling stream 491

Axon Guidance 493

The Intrinsic Navigational Programming of Motor Neurons 494

- Cell adhesion: A mechanism to grab the road 495
- Local and long-range guidance molecules: The street signs of the embryo 496
- Repulsion patterns: Ephrins and semaphorins 496

How Did the Axon Cross the Road? 498

The Travels of Retinal Ganglion Axons 502

- Growth of the retinal ganglion axon to the optic nerve 502
- Growth of the retinal ganglion axon through the optic chiasm 503

Target Selection: “Are We There Yet?” 504

- Chemotactic proteins 504
- Target selection by retinal axons: “Seeing is believing” 505

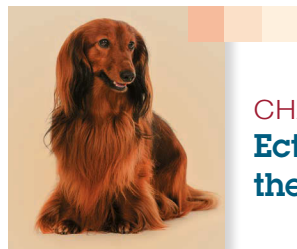
Adhesive specificities in different regions of the optic tectum: Ephrins and Ephs 506

Synapse Formation 508

A Program of Cell Death 509

Activity-dependent neuronal survival 511

Differential survival after innervation: The role of neurotrophins 511



CHAPTER 16 Ectodermal Placodes and the Epidermis 517

Cranial Placodes: The Senses of Our Heads 517

The Dynamics of Optic Development: The Vertebrate Eye 520

Formation of the Eye Field: The Beginnings of the Retina 521

The Lens-Retina Induction Cascade 523

Lens and cornea differentiation 525

Neural retina differentiation 526

The Epidermis and Its Cutaneous Appendages 528

Origin of the Epidermis 528

The Ectodermal Appendages 529

Recombination experiments: The roles of epithelium and mesenchyme 530

Signaling pathways 531

Ectodermal appendage stem cells 533

Coda 537

PART V ■ Building with Mesoderm and Endoderm: Organogenesis



CHAPTER 17 Paraxial Mesoderm: The Somites and Their Derivatives 539

Cell Types of the Somite 542

Establishing the Paraxial Mesoderm and Cell Fates Along the Anterior-Posterior Axis 543

Specification of the paraxial mesoderm 543

Spatiotemporal collinearity of Hox genes determine identity along the trunk 545

Somitogenesis 548

Axis elongation: A caudal progenitor zone and tissue-to-tissue forces 549

The clock-wavefront model 552

Linking the clock-wavefront to Hox-mediated axial identity and the end of somitogenesis 558

Sclerotome Development 560

Vertebrae formation 562

Tendon formation: The syndetome 565

Formation of the dorsal aorta 566

Dermomyotome Development 566

Determination of the central dermomyotome 568

Determination of the myotome 568

An emerging model of neural crest-regulated myogenesis 570

Osteogenesis: The Development of Bones 572

Endochondral ossification 572

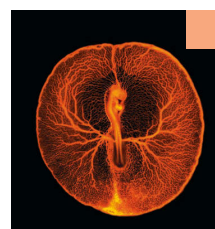
Mechanotransduction and vertebrate bone development 574

Maturation of Muscle 575

Myoblasts and myofibers 575

Satellite cells: Unfused muscle progenitor cells 577

Mechanotransduction in the musculoskeletal system 578



CHAPTER 18 Intermediate and Lateral Plate Mesoderm: Heart, Blood, and Kidneys 581

Intermediate Mesoderm: The Kidney 582

Specification of the Intermediate Mesoderm: Pax8 and Lim1 584

Reciprocal Interactions of Developing Kidney Tissues 585

Mechanisms of reciprocal induction 586

Lateral Plate Mesoderm: Heart and Circulatory System 591

Heart Development 592

A minimalist heart 592

Formation of the heart fields 593

Specification of the cardiogenic mesoderm 595

Migration of the cardiac precursor cells 596
Initial heart cell differentiation 599

Blood Vessel Formation 601

Vasculogenesis: The initial formation of blood vessels 601
Angiogenesis: Sprouting of blood vessels and remodeling of vascular beds 604
Anti-angiogenesis in normal and abnormal development 605

Hematopoiesis: Stem Cells and Long-Lived Progenitor Cells 605

Sites of hematopoiesis 606
The bone marrow HSC niche 608
Hematopoietic inductive microenvironments 609

Coda 610



CHAPTER 19
Development of the Tetrapod Limb 613

Limb Anatomy 613

The Limb Bud 614

Hox Gene Specification of Limb Skeleton Identity 616

From proximal to distal: Hox genes in the limb 616
From fins to fingers: Hox genes and limb evolution 617

Determining What Kind of Limb to Form and Where to Put It 619

Specifying the limb fields 619
Induction of the early limb bud 620

Outgrowth: Generating the Proximal-Distal Axis of the Limb 625

The apical ectodermal ridge 625
Specifying the limb mesoderm: Determining the proximal-distal polarity 627

Turing's model: A reaction-diffusion mechanism of proximal-distal limb development 631

Specifying the Anterior-Posterior Axis 635

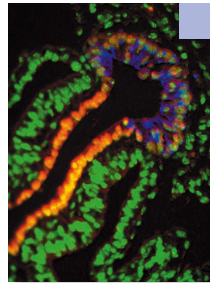
Sonic hedgehog defines a zone of polarizing activity 635
Specifying digit identity by Sonic hedgehog 636
Sonic hedgehog and FGFs: Another positive feedback loop 639
Hox specification of the digits 640
A Turing model for self-organizing digit skeletogenesis 642

Generating the Dorsal-Ventral Axis 644

Cell Death and the Formation of Digits and Joints 645

Sculpting the autopod 645
Forming the joints 646
Continued limb growth: Epiphyseal plates 647
Fibroblast growth factor receptors: Dwarfism 648

Evolution by Altering Limb Signaling Centers 649



CHAPTER 20
The Endoderm: Tubes and Organs for Digestion and Respiration 653

The Pharynx 655

The Digestive Tube and Its Derivatives 657

Specification of the gut tissue 658
Accessory organs: The liver, pancreas, and gallbladder 660

The Respiratory Tube 666

PART VI ■ **Postembryonic Development**



CHAPTER 21
Metamorphosis: The Hormonal Reactivation of Development 671

Amphibian Metamorphosis 672

Morphological changes associated with amphibian metamorphosis 673
Hormonal control of amphibian metamorphosis 675
Regionally specific developmental programs 678

Metamorphosis in Insects 679

Imaginal discs 680
Hormonal control of insect metamorphosis 683
The molecular biology of 20-hydroxyecdysone activity 685
Determination of the wing imaginal discs 688

Metamorphosis of the Pluteus Larva 690



CHAPTER 22 Regeneration 693

Many Ways to Rebuild 694

Hydra: Stem Cell-Mediated Regeneration, Morphallaxis, and Epimorphosis 695

Routine cell replacement by three types of stem cells 696

The head activator 697

The head inhibition gradients 699

Stem Cell-Mediated Regeneration in Flatworms 701

Salamanders: Epimorphic Limb Regeneration 707

Formation of the apical epidermal cap and regeneration blastema 708

Proliferation of the blastema cells: The requirement for nerves and the apical epidermal cap 711

Luring the Mechanisms of Regeneration from Zebrafish Organs 714

Regeneration in Mammals 718



CHAPTER 23 Aging and Senescence 723

Genes and Aging 723

DNA repair enzymes 724

Aging and the insulin signaling cascade 726

The mTORC1 pathway 728

Chromatin modification 728

Random Epigenetic Drift 729

Stem Cells and Aging 730

Exceptions to the Aging Rule 731

PART VII ■ Development in Wider Contexts



CHAPTER 24 Development in Health and Disease: Birth Defects, Endocrine Disruptors, and Cancer 735

The Role of Chance 736

Genetic Errors of Human Development 736

The nature of human syndromes 736

Genetic and phenotypic heterogeneity 738

Teratogenesis: Environmental Assaults on Animal Development 738

Alcohol as a teratogen 741

Retinoic acid as a teratogen 744

Endocrine Disruptors: The Embryonic Origins of Adult Disease 746

Diethylstilbestrol (DES) 747

Bisphenol A (BPA) 749

Atrazine: Endocrine disruption through hormone synthesis 751

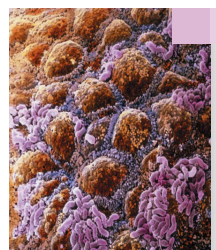
Fracking: A potential new source of endocrine disruption 752

Transgenerational Inheritance of Developmental Disorders 753

Cancer as a Disease of Development 754

Developmental therapies for cancer 758

Coda 759



CHAPTER 25 Development and the Environment: Biotic, Abiotic, and Symbiotic Regulation of Development 763

The Environment as a Normal Agent in Producing Phenotypes 764

Diet-induced polyphenisms 764

Predator-induced polyphenisms 768

Temperature as an environmental agent 771

Polyphenic Life Cycles 773

Larval settlement 773

The hard life of spadefoot toads 774

Developmental Symbioses 775

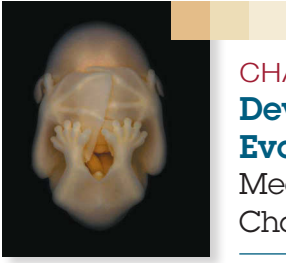
Mechanisms of developmental symbiosis: Getting the partners together 776

The *Euprymna-Vibrio* symbiosis 777

Obligate developmental mutualism 778

Developmental symbiosis in the mammalian intestine 779

Coda 782



CHAPTER 26
Development and Evolution: Developmental Mechanisms of Evolutionary Change **785**

Descent with Modification: Why Animals Are Alike and Different 786

Preconditions for Evolution: The Developmental Structure of the Genome 786

Modularity: Divergence through dissociation 787

Molecular parsimony: Gene duplication and divergence 789

Deep Homology 792

Mechanisms of Evolutionary Change 793

Heterotopy 794

Heterochrony 795

Heterometry 796

Heterotypy 798

Developmental Constraints on Evolution 799

Selectable Epigenetic Variation 801

Genetic assimilation 804

Fixation of environmentally induced phenotypes 806

Coda 807

Glossary G-1

Author Index AI-1

Subject Index SI-1

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 capacitor 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 refigure 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 acknowledgment 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.

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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.

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to accompany **Developmental Biology**, Eleventh Edition

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MARY S. TYLER and RONALD N. KOZLOWSKI

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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

¹More 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 benefited 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 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.

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).

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

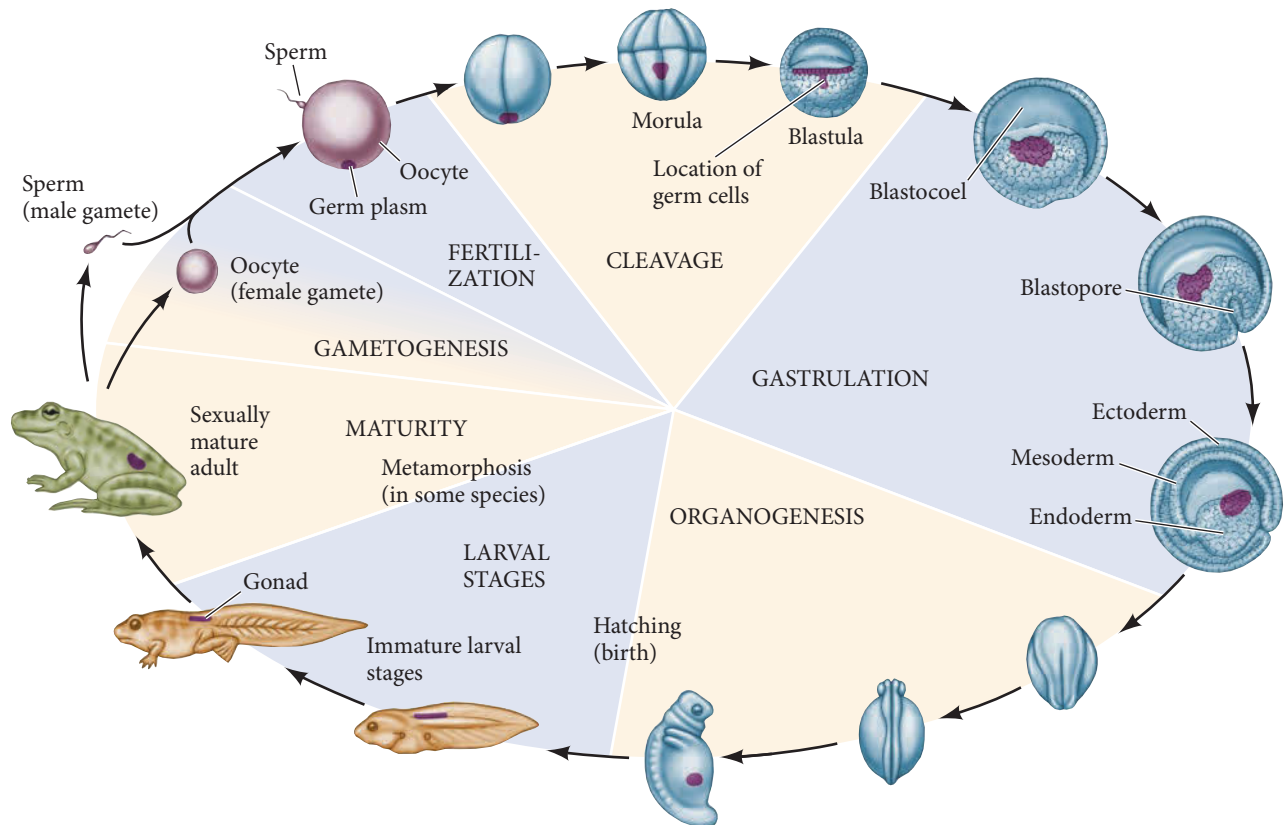


FIGURE 1.1 Developmental history of the leopard frog, *Rana pipiens*. The stages from fertilization through hatching (birth) are known collectively as embryogenesis. The region set aside for producing germ cells is shown in purple. Gametogenesis, which is completed in the sexually mature adult, begins 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.

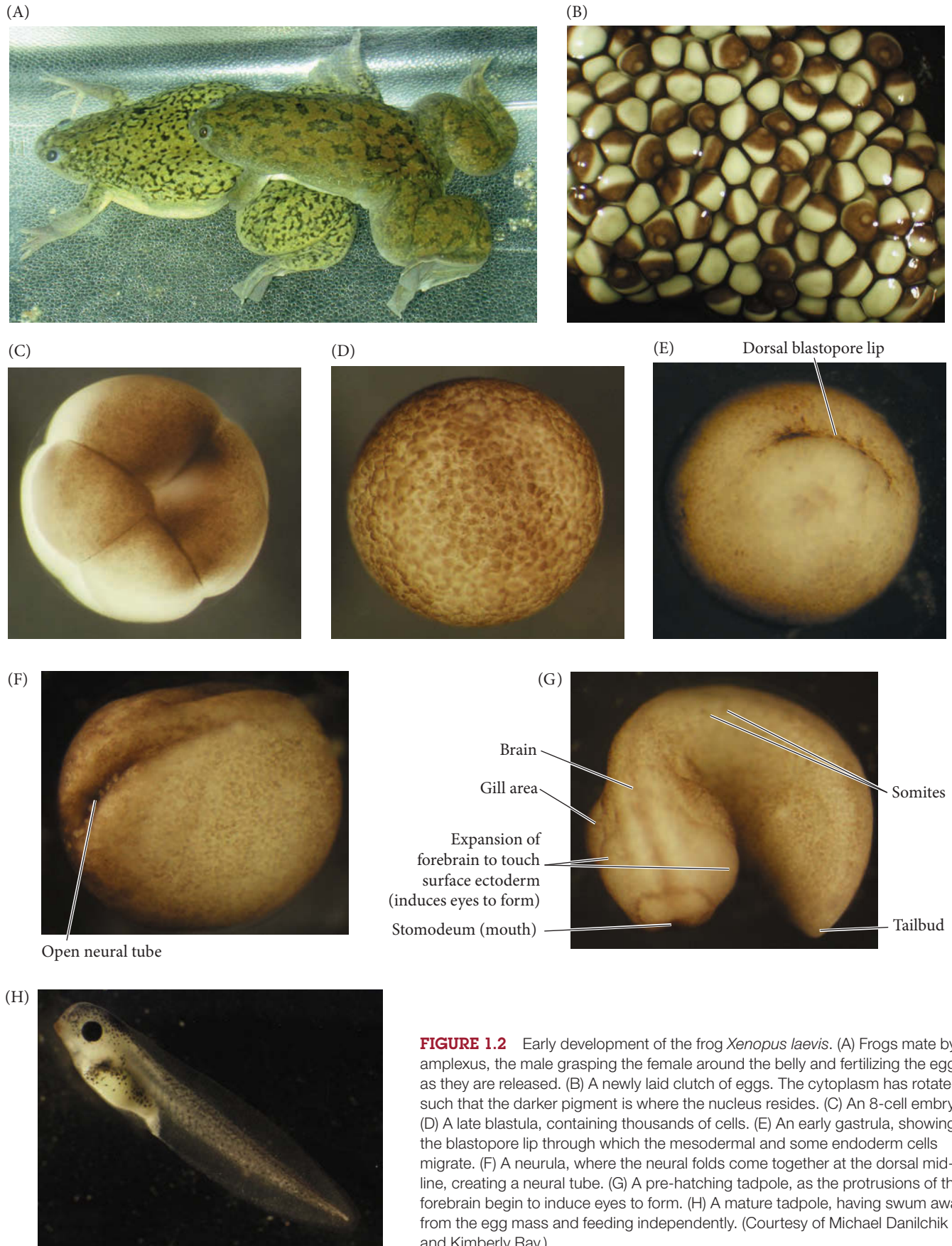


FIGURE 1.2 Early development of the frog *Xenopus laevis*. (A) Frogs mate by amplexus, the male grasping the female around the belly and fertilizing the eggs as they are released. (B) A newly laid clutch of eggs. The cytoplasm has rotated such that the darker pigment is where the nucleus resides. (C)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

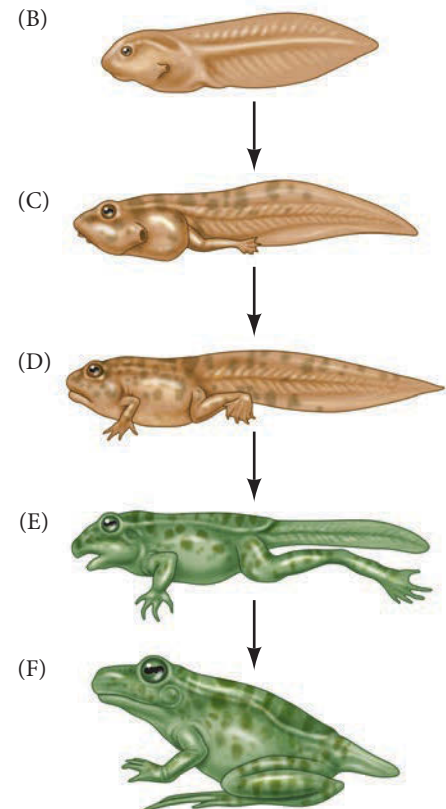
Metamorphosis and gametogenesis

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 unincubated⁴ 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

³Harvey 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.

⁴As 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.