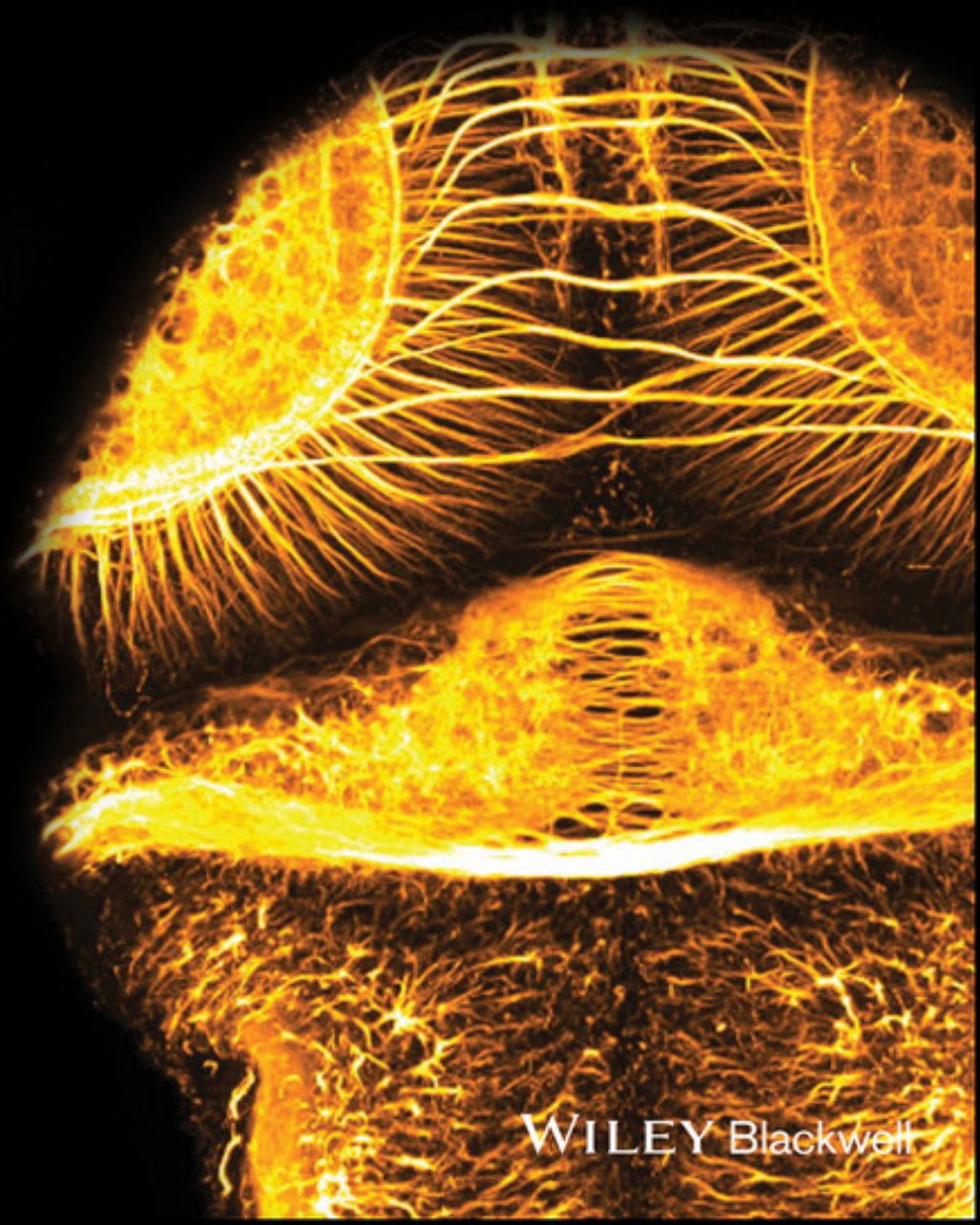
JONATHAN M. W. SLACK · LESLIE DALE

ESSENTIAL DEVELOPMENTAL BIOLOGY

4 | FOURTH EDITION



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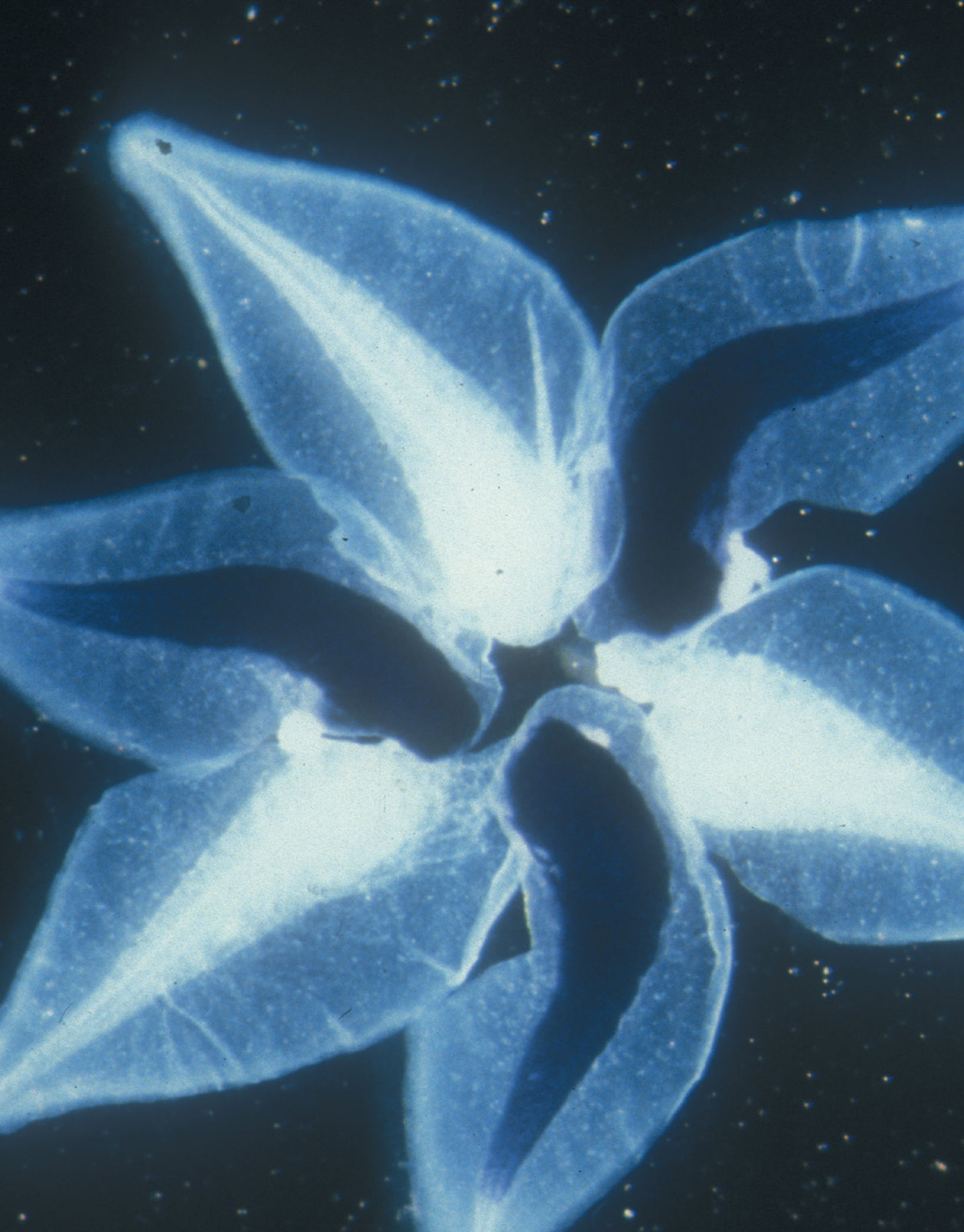
Essential Developmental Biology



4th Edition

Essential Development Biology

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WILEY

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Preface

This book presents the basic concepts and facts relating to the developmental biology of animals. It is designed as a core text for undergraduate courses from the first to the fourth year, and also for first year graduate students. It is suitable for both biologically based and medically oriented courses. A basic knowledge of cell and molecular biology is assumed, but no prior knowledge of development, animal structure, or histology should be necessary.

For this, the fourth edition, the work has two authors. Leslie Dale was head of teaching for cell and developmental biology at University College London and has brought his invaluable teaching experience as well as his daily contact with cutting-edge research to complement the expertise of the senior author, Jonathan Slack. Two technical advances in particular have been incorporated into the text. The first is single cell transcriptome sequencing which provides a completely new way to observe developmental fate and commitment at a molecular level. The second is CRISPR-Cas9, and other methods for targeted genetic manipulation, that have hugely extended the range of what can be done.

The book is arranged in four parts, and the order of topics is intended to represent a logical progression. The first part introduces the basic concepts and techniques. Chapter 2, "How Development Works," is intended as a very brief summary of developmental mechanisms suitable for introductory lectures. We have moved the biochemistry of signaling systems, formerly in the appendix, into Chapter 4. So now the theoretical concepts of experimental embryology are presented together with the molecular pathways that underlie them. Part 1 also contains a new chapter on "Cells into Tissues" (Chapter 6) dealing with the fundamentals of morphogenesis and the underpinning role of cell contacts and the cytoskeleton. In other works, this topic is often fragmented among many individual examples and loses its coherence.

The second part covers the early development of the six main "model organisms," *Xenopus*, zebrafish, chick, mouse, *Drosophila*, and *Caenorhabditis elegans*, up to the stage of the general body plan. For this edition, we have included a new "model" which is the human embryo itself. Of course, it is the animal species that are models for humans, but we now consider that enough is known about human development in its own right to make a separate chapter both necessary and desirable.

The third part deals with organ development, mostly of vertebrates but including also *Drosophila* imaginal discs. It has been fully rewritten and updated, and particular attention is drawn to cases where the molecular basis of human developmental defects is now understood.

The fourth part deals with some topics of high contemporary interest: tissue organization and stem cells, growth, aging and cancer, regenerative medicine, evolution, and regeneration. Regeneration now comes last, in Chapter 24, because we find it is useful to have been exposed to the introduction to animal classification provided by Chapter 23, "Evolution and Development," in order to understand the nature of some of the regeneration models.

Like the previous editions, this new version of *Essential Developmental Biology* differs from its main competitors in four important respects, all of which we feel are essential for effective education.

- It keeps the model organisms separate when early development is discussed. This avoids the muddle that arises all too often when students think that knockouts can be made using *Xenopus* ES cells, or that bindin is essential for mammalian fertilization.
- It avoids considerations of history and experimental priority because students do not care who did something first if it all happened 20–30 years ago.
- It does, however, explain *why* we believe what we do. Understanding does not come from simply memorizing long lists of gene names, so we continue to explain how to investigate developmental phenomena and what sorts of evidence are needed to prove a particular type of result.
- The work is highly focused. In order to keep the text short and concise, we have limited the number of organ systems that are discussed and we have not wandered off into areas such as the development of plants or lower eukaryotes, that may be very interesting but are really separate branches of biology.

The first three editions were very well received by both users and reviewers, and we hope that the fourth edition will make

this book an even more popular choice for undergraduate and graduate-level teaching around the world.

Obstacles to learning

Students sometimes consider developmental biology to be a difficult subject, but this need not be the case so long as certain obstacles to understanding are identified at an early stage. The names and relationships of embryonic body parts are generally new to students, so in this book the number of different parts mentioned is kept to the minimum required for understanding the experiments, and a consistent nomenclature is adopted (e.g. "anterior" is used throughout rather than "rostral" or "cranial").

The competitor texts mix up species and, for example, would typically consider sea urchin gastrulation, *Xenopus* mesoderm induction, and chick somitogenesis in quick succession. This leaves the student unsure about which processes occur in which organisms. In order to avoid confusion, we have kept separate the model organism species in Part 2, and for Part 3 and 4 it is made clear to which organisms particular findings apply.

Although most students do understand genetics in its simple Mendelian form, they do not necessarily appreciate certain key features prominent in developmental genetics. Among these are the fact that one gene can have several mutant alleles with different properties (e.g. loss of function, constitutive, or dominant negative), or that the name of a gene often corresponds to its loss-of-function phenotype rather than its normal function (e.g. the normal function of the *dorsal* gene in *Drosophila* is to promote ventral development!). Furthermore, pathways with inhibitory steps, such as the Wnt pathway, cause considerable trouble because of the difficulty of representing the lack of something in a diagram. Here, these issues are fully explained in the early chapters, with appropriate reinforcement later on. We provide charts showing the state of each component of inhibitory pathways such that the consequences of altering a particular component can be seen at a glance. We also always distinguish clearly between loss-of-function, gain-of-function, and dominant negative mutations.

Gene nomenclature is an awkward problem for a textbook because there are different conventions in use for different model organisms, and between those genes discovered through mutation as opposed to those discovered via biochemistry of the protein product. Here, the species-specific conventions are followed where the text relates to a particular species, but if the text relates a specific gene in more than one species, we use a generic convention with the name italicized and an initial capital letter.

Students usually fail to distinguish between genes and gene products, and should hopefully be encouraged to do so by the use of italics for gene symbols and regular type for proteins. It is also necessary to understand the difference between increasing the expression of a gene product and activating the biochemical function of a product that is already present. Here, we try to make this easier by referring respectively to "upregulation" and "activation" for these two situations, and to "repression" and "inhibition" for the situation where expression or activity is reduced.

We are careful not to adopt the colloquial joining of name and function, as for example in "Notch receptor." This all too easily suggests a receptor *for* Notch rather than the Notch molecule itself, and is a style best avoided.

Finally, we have tried to keep the overall level of detail, in terms of the number of genes, signaling systems, and other molecular components, to the minimum required to explain the workings of a particular process. This often means that various parallel or redundant components are not mentioned, and the latest detail published in *Cell* is omitted.

Learning outcomes

When students have completed a course corresponding to the content of this book, they should be able to understand the main principles and methods of the subject. If they wish to enter graduate school, they should be very well prepared for a graduate program in developmental biology. If they go to work in the pharmaceutical industry, they should be able to evaluate assays based on developmental systems where these are used for the purposes of drug screening or drug development. If they become high school teachers, they should be able to interpret the increasing flow of stories in the media dealing with developmental topics, which are sometimes inaccurate and often sensationalized. Whether the story deals with miracle stem cell cures, human cloning, four-legged chickens, or headless frogs, the teacher should be able to understand and explain the true nature of the results and the real motivation behind the work. It is in all our interests to ensure that the results of scientific research are disseminated widely, but also that they are a source of enlightenment and not of sensation.

Acknowledgements

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About the companion website

This book is accompanied by a companion website.

www.wiley.com/go/essentialdevelopmentalbiology4e

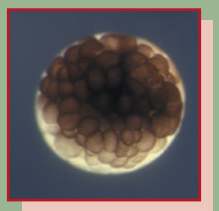
This website includes:

- Animations
- Artwork
- Self-assessment
- Glossary
- Useful websites

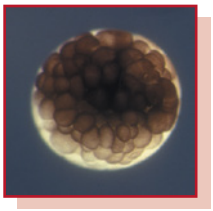
and more

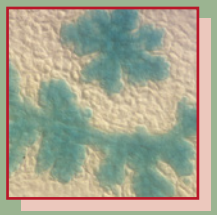
The website is sign-posted throughout the book. Look out for these icons:

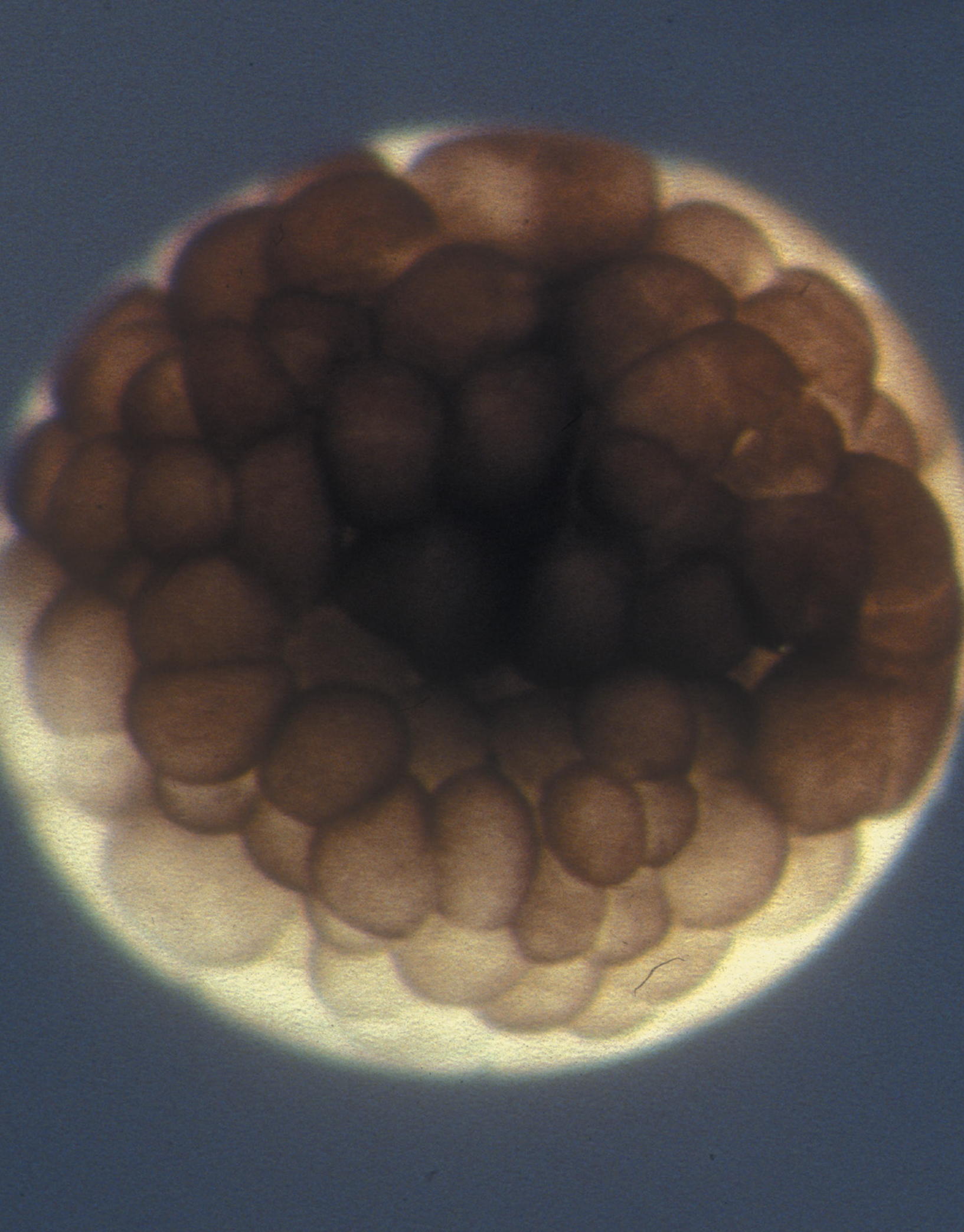
Section 1



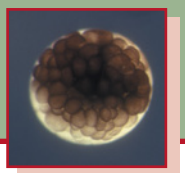
Groundwork







Chapter 1



The excitement of developmental biology

Developmental biology is the science of how biological form changes in time. Development occurs most obviously in the embryo, where the fertilized egg develops into a complex animal containing many cell types, tissues, and body parts. But development also occurs in other contexts, for example during regeneration of missing body parts, during metamorphosis of larval animals to the adult form, and even within our own bodies as the continuous differentiation of new cells.

Developmental biology occupies a unique central position in modern biology. This is because it unites the disciplines of molecular/cellular biology, genetics, and morphology. Molecular and cell biology tell us about how individual genes and cells work. In development this means the operation of intercellular signaling factors, their receptors, intracellular signal transduction pathways, and the transcription factors that regulate gene expression. Genetics tells us directly about the function of an individual gene and how it relates to the activities of other genes. Morphology, or anatomical structure, is both a consequence and a cause of the molecular events. This is because the first processes of development create a simple subdivision of parts in the embryo, which then serves as the basis on which further rounds of signaling and responses can occur, creating a progressively more complex morphology.

So developmental biology is a discipline involving contributions from these three areas of science. When thinking about developmental problems, it is necessary to be able to use concepts from the three areas simultaneously because they are all required to achieve a complete picture.

Where the subject came from

One of the most amazing conclusions of modern research is that the mechanisms of development are very similar for all animals, including humans. This fact has only been known since it has become possible to examine the molecular basis of developmental processes. Before 1980 we knew virtually nothing of these mechanisms, but 40 years later we know a lot and there are several undergraduate textbooks on the subject. Over this period, developmental biology has been one of the most exciting areas of biological research. Each of the components of the modern discipline has its own historic tradition – experimental embryology, developmental genetics, and molecular biology – that eventually became fused together into the present single world view.

Experimental embryology had been going since the late nineteenth century. In its early decades it consisted mainly of microsurgical experiments on embryos of amphibians and sea urchins. These demonstrated the existence of **embryonic induction**: chemical signals that controlled the pathways of development of cells within the embryo. The experiments showed where and when these signals operated, but they could not identify the signals nor the molecular nature of the responses to them.

Developmental genetics has existed since the early twentieth century, but it really flowered in the late 1970s when mass genetic screens were carried out on the fruit fly *Drosophila melanogaster*, in which thousands of mutations affecting development were examined. These **mutagenesis screens** resulted in the identification of a high proportion of the genes that control development, not just in *Drosophila* but in all animals. The curious names that developmental genes often have, even in humans, reflect their original names on the basis of their effects in *Drosophila*.

Molecular biology effectively started with the discovery of the three-dimensional structure of DNA in 1953, and became a practical science of gene manipulation in the 1970s. The key technical innovations were methods for **molecular cloning** to enable single genes to be amplified to a chemically useful

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quantity, methods for **nucleic acid hybridization** to enable the identification of DNA or RNA samples, and methods for **DNA sequencing** to determine the primary structures of genes and their protein products. Once this toolkit had been assembled, it could be applied to a whole range of biological problems, including those of development. It was used initially to clone the developmental genes of *Drosophila*. This turned out to be of enormous importance because most of the key *Drosophila* genes were found to exist also in other animals, and frequently to be controlling similar developmental processes. Molecular biological methods were also applied directly to vertebrate embryos and used to identify the previously mysterious inducing factors and the genes regulated by them.

The application of molecular biology techniques meant that the mechanisms of development could, for the first time, be understood in molecular detail. In particular, the domains of gene activity could be directly visualized using wholemount *in situ* hybridization. It also meant that the path of development could be experimentally altered by the introduction of new genes, or the selective removal of genes, or by an alteration of the regulatory relationships between genes. It also showed that all animals use very similar mechanisms to control their development. This is particularly exciting because it means that we really can learn about human development by understanding how it happens in the fruit fly, zebrafish, frog, or mouse.

Impact of developmental biology

Apart from its intellectual excitement, some areas of developmental biology have had a significant practical impact on society. ***In vitro* fertilization (IVF)** is now a routine procedure and has enabled millions of previously infertile couples to have a baby. It is estimated that about 2–3% of births in developed countries now arise from IVF. Its variants include artificial insemination by donor (AID), egg donation, and storage of fertilized eggs by freezing. In 2010, Robert Edwards (1925–2013) received the Nobel Prize in Physiology/Medicine for introducing this technique. It is less widely appreciated that AID, IVF, embryo freezing, and embryo transfer between mothers are also very important for farm animals. These techniques have been used for many years in cattle to increase the reproductive potential of the best animals.

Developmental biology also led to the understanding that human embryos are particularly sensitive to damage during the period of **organogenesis** (i.e. after the general body plan is formed, and while individual organs are being laid down). The science of **teratology** studies the effects of environmental agents such as chemicals, viral infection, and radiation on embryos. This has led to an awareness of the need to protect pregnant women from the effects of these agents. For example, the statin drugs, used to lower cholesterol levels, can compromise the cholesterol modification of the signaling molecule Sonic Hedgehog. This can lead to a variety of defects in systems dependent on Hedgehog signaling during development: the central nervous system (CNS), limbs, and vertebrae. Although normal doses of statins are unlikely to be teratogenic in humans, this provides a good reason to avoid them during early pregnancy.

Developmental biology is responsible for an understanding of the genetic or chromosomal basis of many human **birth defects**. For example, Down syndrome is due to the presence of an extra chromosome 21, and there exist a number of relatively common abnormalities of the sex chromosomes. These can be detected in cells or DNA taken from the amniotic fluid and form the basis of the **amniocentesis** tests taken by millions of expectant mothers every year. They can also be detected in **chorionic villi**, a part of the placenta derived from the conceptus, which may be sampled in the early stages of pregnancy. It is also now possible to screen for defects in single cells removed from the early conceptus following IVF (**preimplantation diagnosis**). Many other birth defects are due to mutations in specific genes that control development. These may be screened for in the DNA of the parents, or that of the preimplantation conceptus, or that of the chorionic villi, using molecular biology techniques.

Developmental biology research has also led to the identification of several new growth regulatory substances, some of which have entered clinical practice. For example, the hematopoietic growth factors erythropoietin and granulocyte–macrophage colony-stimulating factor (GM-CSF) have both been used for some years to treat patients whose blood cells are depleted by cancer chemotherapy, or for other reasons. Some others, such as the platelet-derived growth factor (PDGF), have been used to assist the healing of wounds.

Developmental biology has also impacted in a major way on other areas of science. This is especially true of the methods for making genetically modified mice, which are now commonly used as **animal models** of human diseases, enabling more detailed study of pathological mechanisms and the testing of new experimental therapies. These are by no means limited to models for genetic disease, as often a targeted mutation in the mouse can mimic a human disease that arises from non-mutational causes.

Last but not least, developmental biology has been the "midwife" of stem cell biology. **Embryonic stem cells** were discovered by developmental biologists, and human embryonic stem cells were first isolated in 1998. These are **pluripotent** cells, which means that any cell type in the body can be obtained from them using methods for **directed differentiation** *in vitro*. These methods depend very largely on the understanding of the normal sequence of signals and responses in the embryo, which has been built up by developmental biologists. It is now also possible to make **induced pluripotent stem cells (iPS cells)** from any individual by overexpressing certain genes in normal cells from the skin or blood. Functional cell types obtained from human pluripotent stem cells, particularly heart muscle and liver, are now

used for safety screening of new drugs. Several clinical trials are investigating the use of cell transplants derived from pluripotent stem cells for the treatment of various diseases, for example retinal pigment epithelium for treatment of macular degeneration, and dopaminergic neurons for the treatment of Parkinson's disease.

Future impact

Although the past impact of developmental biology is considerable, the future impact will certainly be much greater. Some of the applications, particularly those involving human genetic manipulation, may cause some serious ethical and legal problems. These problems will have to be resolved by society as a whole and not just the scientists who are the current practitioners of the subject. For this reason, it is important that an understanding of developmental biology becomes as widespread as possible, because only with an appreciation of the science will people be able to make informed choices.

The scope for assisted reproduction will increase as methods are perfected to create gametes from pluripotent stem cells. This will enable completely infertile people to have children derived from their own iPS cells, although for the foreseeable future the resulting conceptuses will still need to be implanted in the womb of either the biological mother or a surrogate mother to enable development to term.

We can expect to see an extension of **prenatal screening** to encompass the whole variety of single-gene disorders. Although this is welcome as a further step in the elimination of human congenital defects, it also presents a problem. The more tests that are performed on an individual's genetic makeup, the more likely they are to be denied insurance or particular career opportunities because they are found to have a susceptibility to some disease or other.

The application of developmental biology to the production of human cells, tissues, or organs for **transplantation** will certainly expand. There is now the possibility of making grafts from patient-specific iPS cells, which will be a perfect immunological match for the patient. Stem cell technology is becoming fused with the methods for **tissue engineering**, which can potentially generate more complex tissues and organs starting with the constituent cell types. This involves the production of novel types of three-dimensional extracellular matrix, or **scaffold**, on which the cells grow and with which they interact. Tissue engineering will need more input from developmental biology in order to be able to create tissues containing several interacting cell types, or tissues with appropriate vascular and nerve supplies. Stem cell technology will also embrace **gene therapy**, which relates to the introduction or modification of specific genes for therapeutic purposes. So transplants derived from stem cells may also carry specific genetic modifications to rectify problems suffered by the patient.

Finally, we should not overlook the likely applications of developmental biology to agriculture. With farm animals the possibilities are likely to be limited by a public wish to retain a "traditional" appearance for their cows, pigs, sheep, and poultry, but already technologies have been developed to produce rapidly growing fish, pharmaceuticals in the milk of sheep, and vaccines in eggs, and other opportunities will doubtless present themselves in the future.

Further reading

Useful websites

Society for Developmental Biology – Education section: http://www.sdbonline.org/education

British Society for Developmental Biology – Advocacy: http://bsdb.org/advocacy/

Textbooks, mainly descriptive

Gilbert, S.F. & Raunio, A.M., eds. (1997) *Embryology: Constructing the Organism*. Sunderland, MA: Sinauer Associates.

Hildebrand, M. & Goslow, G.E. (2001) *Analysis of Vertebrate Structure*, 5th edn. New York: John Wiley & Sons.

Carlson, B.M. (2019) *Human Embryology and Developmental Biology*, 6th edn. Philadelphia: Elsevier Saunders.

Schoenwolf, G., Bleyl, S., Brauer, P. et al. (2021) *Larsen's Human Embryology*, 6th edn. Philadelphia: Elsevier Saunders.

Textbooks, mainly analytical

Wolpert, L., Tickle, C.A., Martinez Arias, A. (2019) *Principles of Development*, 6th edn. Oxford: Oxford University Press.

Barresi, M.J.F. & Gilbert, S.F. (2020) *Developmental Biology*, 12th edn. Sunderland, MA: Sinauer Associates.

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Ferretti, P., Copp, A., Tickle, C. et al. (2006) *Embryos, Genes and Birth Defects*. Chichester, UK: Wiley.

Gearhart, J. & Coutifaris, C. (2011) In vitro fertilization, the Nobel Prize, and human embryonic stem cells. *Cell Stem Cell* **8**, 12–15.

Araki, M. & Ishii, T. (2014) International regulatory landscape and integration of corrective genome editing into in vitro fertilization. *Reproductive Biology and Endocrinology* **12**, 1–12.

Milunsky, A. & Milunsky, J.M., eds. (2016) *Genetic Disorders and the Fetus: Diagnosis, Prevention, and Treatment*, 7th edn. Hoboken, NJ: Wiley-Blackwell.

Lu, L.N., Lv, B., Huang, K. et al. (2016) Recent advances in preimplantation genetic diagnosis and screening. *Journal of Assisted Reproduction and Genetics* **33**, 1129–1134.

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Gjorevski, N., Ranga, A. & Lutolf, M.P. (2014) Bioengineering approaches to guide stem cell-based organogenesis. *Development* **141**, 1794–1804.

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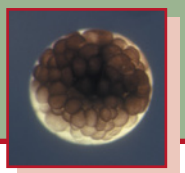
Trounson, A. & DeWitt, N.D. (2016) Pluripotent stem cells progressing to the clinic. *Nature Reviews Molecular Cell Biology* **17**, 194–200.
Shafiee, A. & Atala, A. (2017) Tissue engineering: toward a new era of medicine, in: Caskey, C.T. (ed.), *Annual Review of Medicine*, vol. 68. Palo Alto, CA: Annual Reviews, pp. 29–40.
Slack, J.M.W. (2018) *The Science of Stem Cells*. Hoboken, NJ: Wiley-Blackwell.
Dunbar, C.E., High, K.A., Joung, J.K. et al. (2018) Gene therapy comes of age. *Science* **359**, eaan4672.

Chapter 2



How development works

Some of the basic processes and mechanisms of embryonic development are now quite well understood, others are not. This chapter will give a summary of how development works to the extent that we currently understand it. Evidence for why we believe that the mechanisms are like this, and many examples of developmental processes in specific organisms, will be presented in later chapters. Further information about the genes and molecules that are mentioned by name will be found in Chapter 4.

Embryonic development involves the conversion of a single cell, the fertilized egg, into a complex organism consisting of many anatomical parts. We can break down the complexity of what happens by considering it as five types of process:

1 **Regional specification** deals with how pattern appears in a previously similar population of cells. For example, most early embryos pass through a stage called the **blastula** or **blastoderm** at which they consist of a featureless ball or sheet of cells (Fig. 2.1). The cells in different regions need to become programmed to form different body parts such as the head, trunk, and tail. The initial steps usually involve regulatory molecules deposited in particular positions within the fertilized egg (**determinants**). The later steps usually involve intercellular signaling events, known as **embryonic inductions**, which lead to the upregulation of different combinations of developmental control genes in each zone of cells.

2 Cell **differentiation** refers to the mechanism whereby different sorts of cells arise. There are several hundred different specialized cell types in a vertebrate body, ranging from epidermis to thyroid epithelium, lymphocyte, or neuron. Each cell type owes its special character to the presence of particular proteins encoded by particular genes. The study of cell differentiation deals with the way in which these genes are turned on and how their activity is subsequently maintained. Cell differentiation continues throughout life in regions of persistent cell turnover fed by stem cells.

3 **Morphogenesis** refers to the cell and tissue movements that give the developing organ or organism its shape in three dimensions. This depends on the dynamics of the **cytoskeleton** and on the mechanics and viscoelastic properties of cells. Some morphogenetic processes persist into adult life in regions of tissue renewal.

4 **Growth** refers both to the overall increase of size of the organism, and to the control of proportion between body parts. Although more familiar to the lay person than other aspects of development, it is still less well understood in terms of molecular mechanisms.

5 Somehow the component processes of development are coordinated in time. But **developmental time** remains the most mysterious aspect of the process. We know that different species develop at different rates but we do not know why. In this area there are serious gaps in our knowledge.

Ultrashort summary

The following provides a quick summary of how development works. The remainder of this chapter will explore some basic developmental processes in more detail. Later chapters will explain how these processes work in specific model organisms, or situations of organ development, and provide experimental evidence for the basic models.

Male and female **gametes** develop and undergo **meiosis**, thus halving their **chromosome** number to one copy of each chromosome. The resultant sperm and egg fuse in the process of

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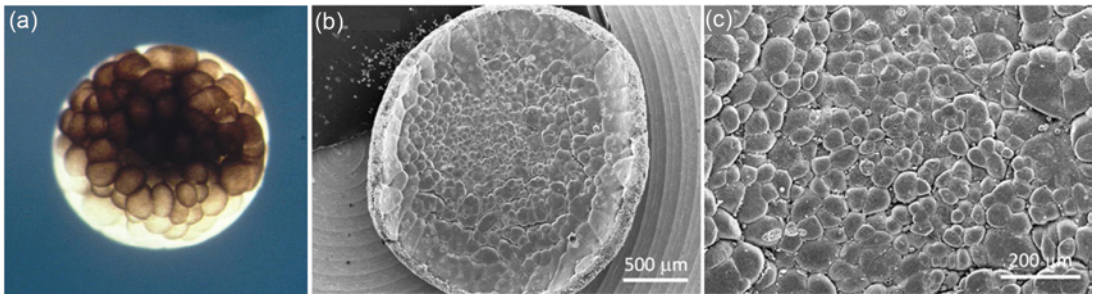

Fig. 2.1 (a) Blastula of a *Xenopus* embryo. (b,c) Blastoderm of a chick embryo. Scanning electron micrographs at low and high power. Sources: (a) Jonathan Slack; (b,c) Nagai et al. (2015). Development. 142, 1279–1286.

fertilization to form a fertilized egg, or **zygote**. This undergoes a period of **cleavage** divisions to form a ball or sheet of similar cells called a **blastula** or **blastoderm**. Cleavage divisions are typically rapid and involve no growth, so the daughter cells are half the size of the mother cell and the whole embryo stays about the same size. A series of morphogenetic movements, called **gastrulation**, converts the original cell mass into a three-layered structure consisting of multicellular sheets called **ectoderm**, **mesoderm**, and **endoderm**, which are known as **germ layers**. During cleavage and gastrulation, the first regional specification processes occur. In addition to the formation of the three germ layers themselves, these processes often generate **extraembryonic** tissues, needed for support and nutrition, and establish differences of commitment between future **anteroposterior** body regions (head, trunk, and tail).

Regional specification is initiated by the presence of **cytoplasmic determinants** in one part of the zygote, which become inherited by the cells that form from this region. This region becomes a signaling center and its cells emit an inducing factor (Figs 2.2 and 2.3). Because it is produced in one place, diffuses away, and decays, the inducing factor forms a concentration gradient, high near the source cells and low further away. The remaining cells of the embryo, which do not contain the determinant, are competent to respond to different concentrations of the inducing factor by upregulating particular developmental control genes. So a series of zones becomes set up, arranged at progressively greater distance from the signaling center established by the determinant. In each zone a different combination of developmental control genes becomes turned on. These encode **transcription factors**, which upregulate new combinations of gene activity in each region. Some of these regions will eventually become new signaling centers, emitting inducing factors different from that emitted by the first center.

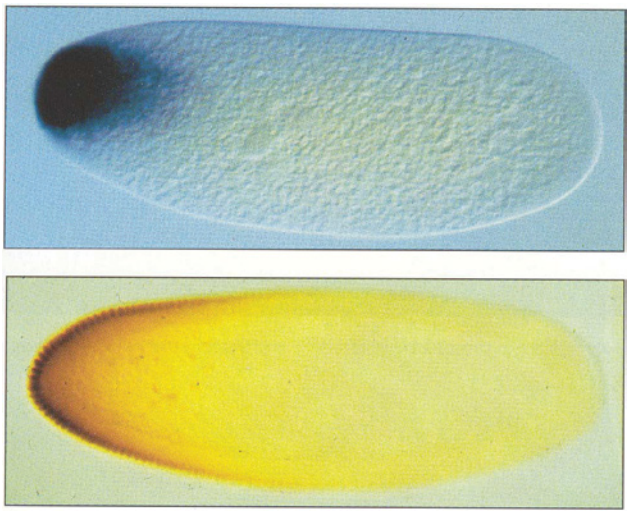

Fig. 2.3 A determinant in the *Drosophila* egg which releases an inducing signal. (a) The determinant is the mRNA for *bicoid* localized at the anterior end (*in situ* hybridization: blue). (b) The inducing signal is a gradient of the bicoid protein (immunostaining: brown). Source: Ephrussi and Johnston (2004). Cell. 116, 143–152.

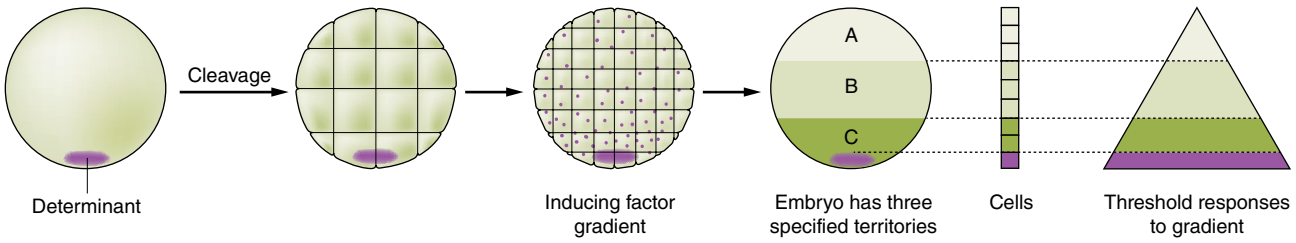

Fig. 2.2 Generation of complexity from a simple beginning. This embryo has a cytoplasmic determinant at the lower end which acts as the source of a morphogen. Genes controlling the formation of two territories, B and C, are upregulated at appropriate threshold concentrations. Territory A is the default that arises in the absence of any inducing factor.

It may seem that all embryos would have to be radially symmetrical, around the axis defined by the direction of diffusion of the first inducing factor. Some embryo types do have radial symmetry, but bilateral symmetry is more usual in animal development. This arises from the fact that there is normally another determinant, off center from the first, and so the initial subdivision of the cells arises from two nonparallel signals. This naturally generates an initially bilateral pattern of territories (Fig. 2.4). Determinants occur in all animal zygotes. In some cases they consist of specific RNA or protein deposited in some part of the egg during oogenesis. In other cases, they become localized as a result of a **symmetry-breaking** process that segregates some substances to one region of the zygote and other substances to other regions (Fig. 2.5). It is this process of symmetry breaking that explains why a spherically symmetrical or radially symmetrical egg can nonetheless initiate the establishment of an internal pattern of structures.

Among the earliest developmental commitments are those responsible for the formation of the three germ layers, **ectoderm**, **mesoderm**, and **endoderm**. These are each associated with the expression of specific transcription factors. Among other functions, these transcription factors upregulate expression of genes conferring specific adhesive and motility properties on the cells in which they are active. Because of these different morphogenetic properties, the cells of each germ layer move to form sheets such that the ectoderm ends up on the outside, mesoderm in the middle, and endoderm on the inside (Fig. 2.6). The morphogenetic movements that result in the positioning of the three germ layers are collectively called **gastrulation**. Morphogenetic movements not only change the shape and structure of the embryo, but by bringing cell sheets into new spatial relationships they also make possible new cycles of signaling and response between these cell populations.

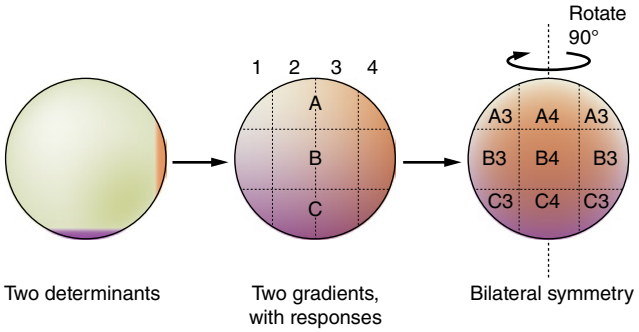

Fig. 2.4 Generation of bilateral symmetry with two determinants. Two gradients (purple and orange) partition the embryo into territories along two axes. The resulting embryo has territories arranged symmetrically around a median plane.

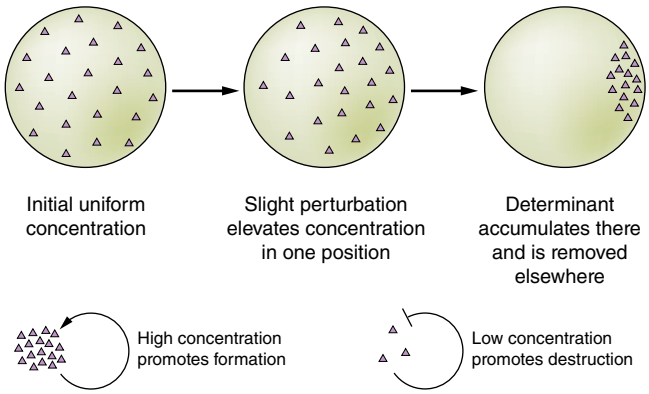

Fig. 2.5 Localization of a determinant by a symmetry-breaking process.

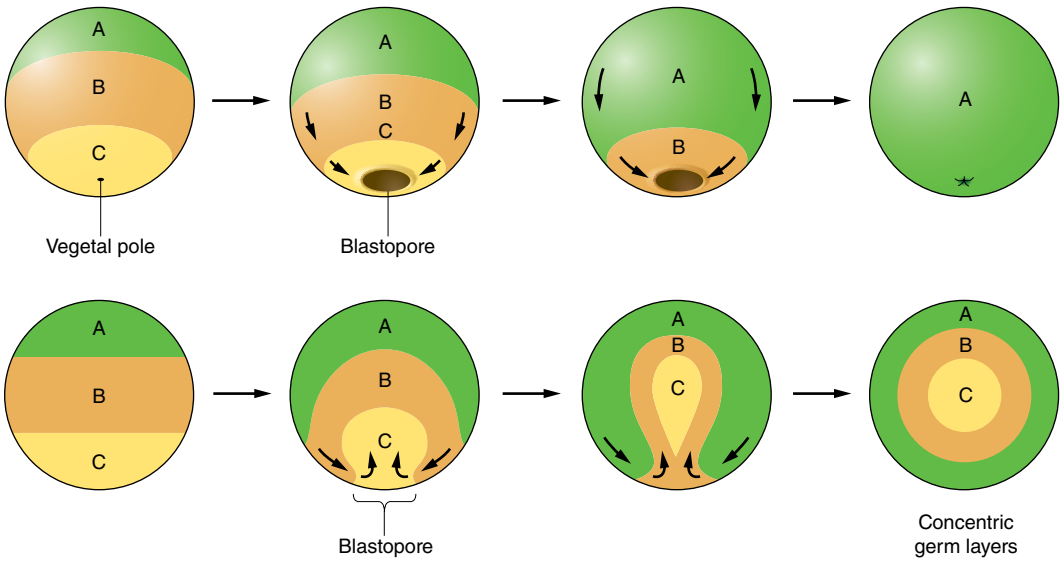

Fig. 2.6 Gastrulation movements: the upper pictures show a surface view and the lower pictures show sections through the embryo. In this very simple example, the C territory invaginates through the lower (or vegetal) pole, followed by the B territory, so that the three territories end up in a concentric arrangement. In reality, gastrulation movements vary considerably between different types of animal.

In the development of a typical embryo there will be many more cycles of the same types of event, involving secretion of an inductive signal by a signaling center and responses to it by cells of the surroundings. Each new territory of cells, with a specific combination of genes active, has a specific competence to respond to inductive signals because of the presence of particular cell surface receptors, particular signal transduction pathways, and particular developmental control genes poised for upregulation once the signal has been received. The number of different types of inducing factor is relatively small, but because competence may change in time the cell populations can respond to the same signals in different ways at different times. For this reason the final complexity that can be built up by successive rounds of inductive signals and responses, together with morphogenetic movements, is very large.

Growth is mostly autonomous. For each territory of cells, the growth rate is controlled by the combination of genes that are active. Free-living embryos do not grow in mass as they have no external food supply. But embryos fed by a placenta or extraembryonic yolk supply can grow very fast, and changes to relative growth rate between parts in these organisms help to produce the final overall anatomy.

The whole process needs to be coordinated in time. For example, it will not work if the secretion and diffusion of inducing factors is not on a time scale consistent with the perception of the signals and the activation of target genes. How this is controlled is still not understood. There may be a master clock able to communicate with all parts of the embryo that controls the course of events, or timing may depend simply on the intrinsic tempo of local causal sequences of events. Recent results suggest that the developmental tempo of different organisms correlates with their overall rate of protein degradation.

Gametogenesis

Sexual reproduction really starts with the formation of the gametes. By definition the male gamete is small and motile and called a spermatozoon (sperm), and the female gamete is large and immotile and called an **egg** or ovum. Each gamete contributes a **haploid** (1n) chromosome set so the zygote is **diploid** (2n), containing a maternal- and a paternal-derived copy of each chromosome.

The gametes are formed from **germ cells** in the embryo, which are referred to collectively as the **germ line**. They comprise cells that will or can become the future gametes, and all other cells are referred to as the **somatic** tissues or soma. Germ cells should not be confused with the three germ layers, and are often formed separately at a very early stage of development. The importance of the germ line is that its genetic information can be passed to the next generation, while that of the soma cannot. A mutation occurring in the DNA of germ cells will be carried to the next generation if the mutated gametes generate mutated zygotes. By contrast, a somatic mutation may occur in a cell at any stage of development and may be important in the life of the individual animal, but it cannot affect the next generation.

It is often the case that the future germ cells become committed to their fate at an early stage of animal development. In some cases there is a cytoplasmic determinant present in the egg that programs cells that inherit it to become germ cells. This is associated with a visible specialization of the cytoplasm called **germ plasm**. It occurs in *Caenorhabditis elegans*, where the cells inheriting the **polar granules** become the P lineage and thereafter the germ cells. It occurs in *Drosophila*, where cells inheriting the **pole plasm** become pole cells and later germ cells. It also occurs in *Xenopus*, where there is a localized germ plasm rich in mitochondria. In other species there may be no visible germ plasm in the egg but germ cells are still formed at a relatively early stage of development.

During embryonic development, germ cells undergo a period of multiplication and will often undergo a migration from the site of their formation to the **gonad**, which may be some distance away. The gonad arises from mesoderm and is initially composed entirely of somatic tissues. After the germ cells arrive they become fully integrated into its structure, and in postembryonic life undergo gamete formation or **gametogenesis**. At some stage in mid-development the key decision of **sex determination** is made, and the gonad becomes determined to become either an ovary or a testis. The molecular mechanism of this is, somewhat surprisingly, different for each of the principal experimental model species, so it will not be described in this chapter. But the upshot is that in the male the germ cells become sperm and in the female they become eggs. Unlike the other model organisms, *C. elegans* is normally a **hermaphrodite** and the germ cells produce both sperm and eggs in the same individual. However, there are also male individuals of *C. elegans*, and the sex determination mechanism controls the male–hermaphrodite decision rather than a male–female decision.

Meiosis

The critical cellular event in gamete production is **meiosis**. This is a modified type of cell cycle in which the number of chromosomes is reduced by half (Fig. 2.7). As in **mitosis**, meiosis is also preceded by a DNA replication phase in which each chromosome becomes replicated to form two identical sister **chromatids**, so the process starts with the nucleus possessing a total DNA content of four times the haploid complement. In mitosis the sister chromatids segregate into two identical diploid daughter cells. But meiosis involves two successive cell divisions. In the first the homologous chromosomes, which are the equivalent chromosome derived from mother and father, pair with each other. At this stage the chromosomes

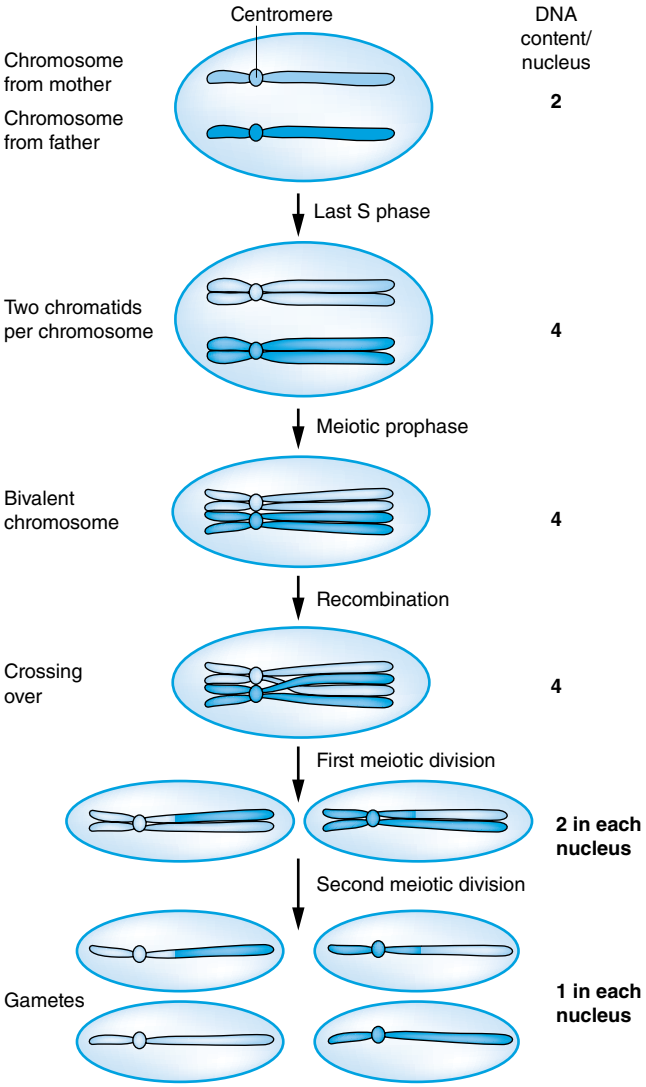

Fig. 2.7 Behavior of chromosomes during meiosis. The DNA content per nucleus is shown at each stage.

are referred to as **bivalents**, and each consists of four chromatids, two maternal-derived and two paternal-derived. **Crossing over** can occur between these chromatids, bringing about recombination of the **alleles** (gene variants) present at different loci. Hence, alleles present at two different loci on the same chromosome of one parent may become separated into different gametes and be found in different offspring. The frequency with which alleles on the same chromosome are separated by recombination is roughly proportional to the physical separation of the loci, and this is why the measurement of recombination frequencies is the basis of genetic mapping. Recombination can also occur between sister chromatids but here the loci should all be identical because they have just been formed by DNA replication, so there are no genetic consequences.

In the first meiotic division the four-stranded bivalent chromosomes separate into homologous pairs, which are segregated to the two daughter cells. There is no further DNA replication, and in the second meiotic division the two chromatids of each chromosome become separated into individual gametes.

It should be noted that the terms **haploid** (1n) and **diploid** (2n) are normally used to refer to the number of homologous chromosome sets in the nucleus rather than the actual amount of DNA. After DNA replication a nucleus contains twice as much DNA as before, but retains the same ploidy designation. The DNA content at each stage of meiosis is shown in Figs 2.7 and 2.8.

Oogenesis

The process of formation of eggs is called **oogenesis** (Fig. 2.8). Following sex determination to female, the germ cells become **oogonia**, which continue mitotic division for a period. After the final mitotic division, the germ cell becomes known as an **oocyte**. It is called a **primary oocyte** until completion of the first meiotic division, and a **secondary oocyte** until completion of the second meiotic division. After this it is known as an unfertilized **egg** or ovum. In all the vertebrate organisms considered in this book, fertilization occurs before completion of the second division, so it is technically an **oocyte** rather than an **egg** that is being fertilized. However, the term "egg" is often used rather loosely to refer to oocytes, fertilized ova, and even early embryos.

Eggs are larger than sperm and the process of oogenesis involves the accumulation of materials in the oocyte. Usually, the primary oocyte is a rather long-lived cell that undergoes a considerable increase in size. Its growth may be assisted by the absorption of materials from the blood, such as the **yolk** proteins of fish or amphibians that are made in the liver. It may also be assisted by direct transfer of materials from other cells. This is seen in *Drosophila*, where the last four mitoses of each oogonium produce an egg chamber containing one oocyte and 15 **nurse cells**. The nurse cells then produce materials that are exported to the oocyte through cytoplasmic bridges. Animals that produce a lot of eggs usually maintain a pool of oogonia throughout life capable of generating more oocytes. Mammals differ from this pattern as they produce all their primary oocytes before birth. In humans, no more oocytes are produced after the seventh month of gestation, and the primary oocytes then remain dormant until puberty.

Ovulation refers to the resumption of the meiotic divisions and the release of the oocyte from the ovary. It is provoked by hormonal stimulation and involves a breakdown of the oocyte nucleus (the **germinal vesicle**) and the migration of the cell-division spindle to the periphery of the cell. The meiotic divisions do not divide the oocyte into two halves, but instead result in the budding off of small **polar bodies**. The first meiotic

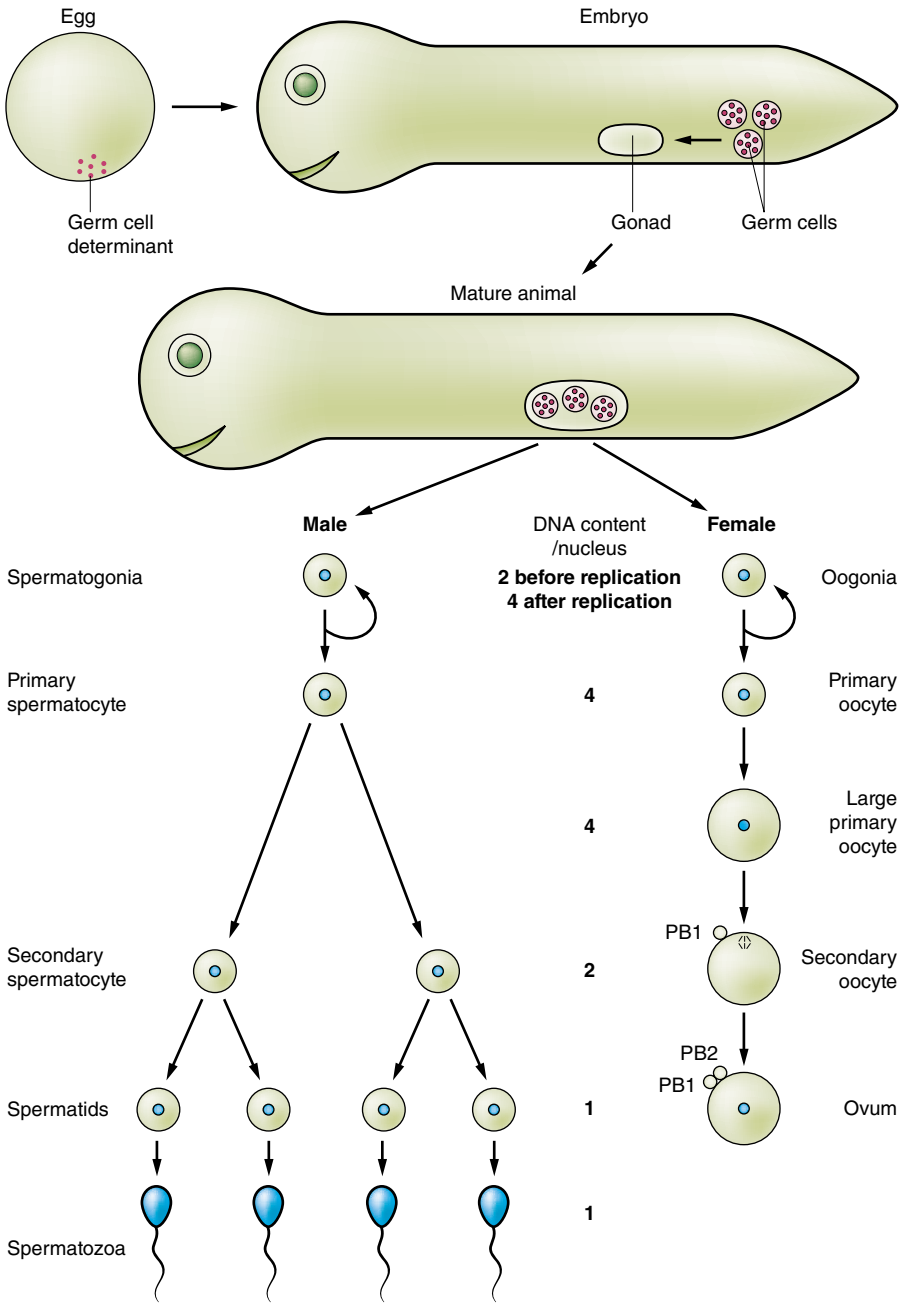

Fig. 2.8 Typical sequence of gametogenesis. The germ cells are initially formed from a cytoplasmic determinant, and during development they migrate to enter the gonad. Spermatogenesis generally results in the production of four haploid sperm per meiosis. Oogenesis generally results in the formation of one egg and two polar bodies (PB1 has the same chromosome number but twice the DNA content as PB2).

division divides the primary oocyte into the secondary oocyte and the first polar body, which is a small projection containing a replicated haploid chromosome set (i.e. 1n information content and 2x DNA content). The second meiotic division divides the secondary oocyte into an egg and a second polar body, which consists of another small projection enclosing a haploid chromosome set (in this case 1n information content and 1x DNA content). The polar bodies soon degenerate and play no further role in development.

Spermatogenesis

If the process of sex determination yields a male then the germ cells undergo spermatogenesis (Fig. 2.8). Mitotic germ cells in the testis are known as **spermatogonia**. Some of these are **stem cells** that can produce more of themselves and also produce **progenitor cells**, which divide a number of times before differentiation into sperm. After the last mitotic division the male germ cell is known as a primary spermatocyte. Meiosis is

equal, the first division yielding two secondary spermatocytes and the second division yielding four spermatids, which mature to become motile spermatozoa.

Early development

Fertilization

The process of fertilization differs considerably between animal groups but there are a few common features. When the sperm fuses with the egg there is a fairly rapid change in egg structure that prevents the fusion of any further sperm. This is called a block to **polyspermy**. Fusion activates the inositol trisphosphate signal transduction pathway (see Chapter 4) resulting in a rapid increase in intracellular calcium. This causes exocytosis of **cortical granules** whose contents form, or contribute to, a fertilization membrane; and also triggers the metabolic activation of the egg, increasing the rate of protein synthesis and, in vertebrates, restarting the second meiotic division. The calcium may, in addition, trigger cytoplasmic rearrangements positioning determinants that are important for the future regional specification of the embryo. For example, dorsal localization of components of the Wnt pathway in *Xenopus* as well as polar granule segregation in *C. elegans* occur in this manner. The sperm and egg pronuclei fuse to form a single diploid nucleus, and at this stage the fertilized egg is known as a **zygote**.

Cleavage

A generalized sequence of early development is shown in Fig. 2.9. A typical zygote of an animal embryo is small, spherical, and polarized along the vertical axis. The upper hemisphere, usually carrying the polar bodies, is called the **animal hemisphere**, and the lower hemisphere, rich in yolk, the **vegetal hemisphere**. The early cell divisions are called **cleavages**. They differ from normal cell division in that there is no growth phase between successive divisions. So each division partitions the mother cell into two half-size daughters (Fig. 2.10). The products of cleavage are called **blastomeres**.

Cell division without growth can proceed for a considerable time in free-living embryos which lack an extracellular yolk mass. Embryos that do have some form of food supply – either mammals that are nourished by the mother, or egg types with a large yolk mass such as birds and reptiles – only undergo a limited period of cleavage at the beginning of development and then commence true growth. In many species, the embryo's own genome remains inactive during part or all of the cleavage phase, and protein synthesis is directed by messenger RNA transcribed during oogenesis (**maternal mRNA**). This is the stage of genetic **maternal effects** because the properties of the cleavage-stage embryo depend on the genotype of the mother and not on that of the embryo itself (see Chapter 3).

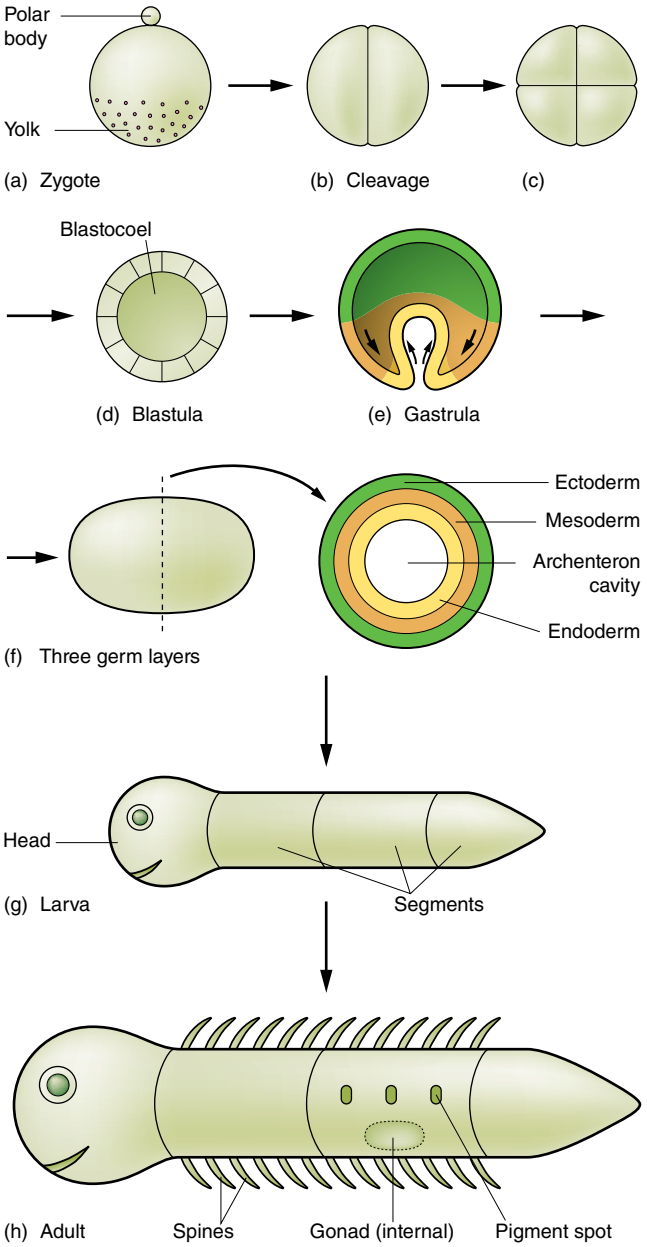

Fig. 2.9 A generalized sequence of early development, comprising cleavage, gastrulation, body plan formation, and differentiation of structures.

Different animal groups display different types of cleavage (Fig. 2.11), and this is controlled to a large extent by the amount of yolk in the egg. Where there is a lot of yolk, as in an avian egg, the cytoplasm is concentrated near the animal pole and only this region cleaves into blastomeres, with the main yolk mass remaining acellular. This type of cleavage is called **meroblastic**. Where cleavage is complete, dividing the whole egg into blastomeres, it is called **holoblastic**. Holoblastic cleavages are often somewhat unequal, with the blastomeres in the yolk-rich vegetal hemisphere being larger (**macromeres**), while those in the animal hemisphere are smaller (**micromeres**). Each

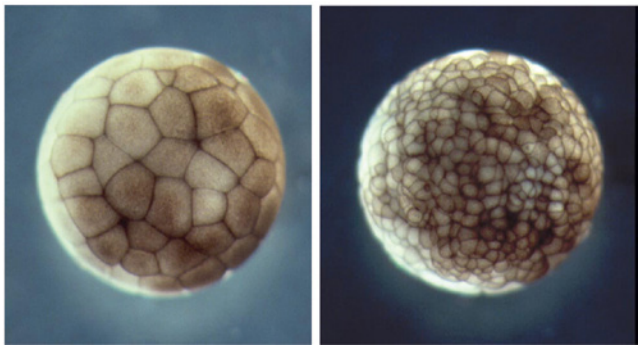

Fig. 2.10 A cleaving embryo of an axolotl. As cell division takes place, the cells become smaller and more numerous so the embryo remains the same size. Source: Jonathan Slack.

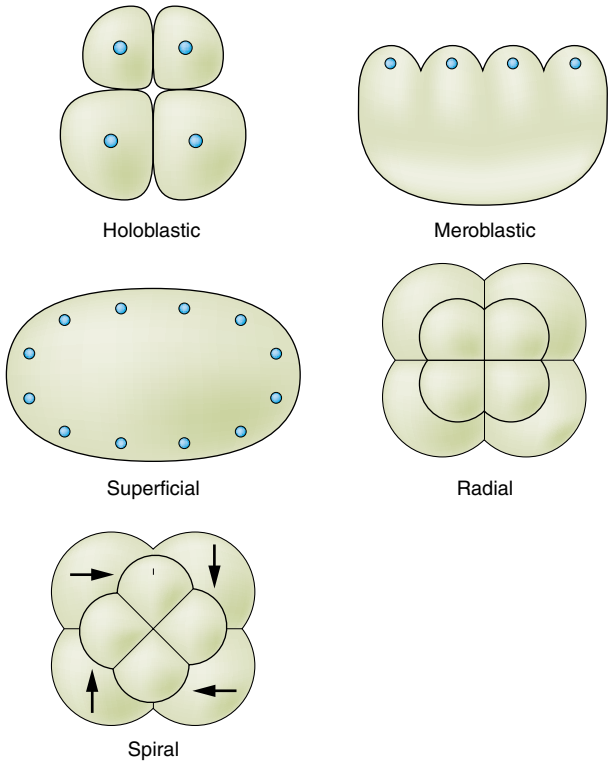

Fig. 2.11 Different types of cleavage found in animal embryos.

animal class or phylum tends to have a characteristic mode of early cleavage, and these can be classified by the arrangement of the blastomeres into such categories as radial (echinoderms), bilateral (ascidians), and rotational (mammals). An important type is the **spiral cleavage** shown by most annelid worms, molluscs, and flatworms. Here, the macromeres cut off successive tiers of micromeres, first in a right-handed sense when viewed from above, then another tier in a left-handed sense, and so on. Most insects and some crustaceans show a special type of cleavage called **superficial cleavage**. Here only the nuclei divide and there is no cytoplasmic cleavage at the early stages. Thus, the early embryo becomes a **syncytium** consisting of many nuclei suspended within the same body of cytoplasm. At a certain stage the nuclei migrate to the periphery, and shortly afterwards cell membranes grow in from the outer surface of the embryo and surround the nuclei to form an epithelium.

During the cleavage phase a cavity usually forms in the center of the ball of cells, or under the sheet of cells in the case of meroblastic cleavage. This expands due to uptake of water and becomes known as the **blastocoel**. At this stage of development the embryo is called a **blastula** or **blastoderm**. The cells often adhere tightly to one another, being bound by cell adhesion molecules called cadherins, and will usually have a system of **tight junctions** forming a seal between the external environment and the internal environment of the blastocoel.

Gastrulation

Following the formation of the blastula, all animal embryos show a phase of cell and tissue movements called **gastrulation**, which converts the simple ball or sheet of cells into a three-layered structure known as the **gastrula**. The details of the morphogenetic movements of gastrulation can vary quite a lot, even between related animal groups (Fig. 2.12), but the outcome is similar. The three tissue layers formed during gastrulation are called **germ layers**, but these should not be confused with **germ cells**. Conventionally, the outer layer is known as the **ectoderm** and later forms the skin and nervous system; the middle layer is the **mesoderm** and later forms the muscles, connective tissue, excretory organs, and gonads; and the inner layer is the **endoderm**, later forming the epithelial tissues of the gut. The **germ cells** have usually appeared by the stage of gastrulation and are not regarded as belonging to any of the three germ layers.

After the completion of the major body morphogenetic movements, most types of animal embryo have reached the general **body plan** stage, at which each major body part is present as a region of committed cells but is yet to differentiate internally. This stage is often called the **phylotypic stage**, because it is the stage at which different members of an animal group, not necessarily a whole phylum, show maximum similarity to each other (see Chapter 23). For example, all vertebrates show a phylotypic stage at the **tailbud** stage when they have a notochord, neural tube, paired somites, branchial arches, and tailbud (Fig. 2.13). All insects show a phylotypic stage at the **extended germ band** stage when they show six head segments, three appendage-bearing thoracic segments, and a variable number of abdominal segments.

Axes and symmetry

In order that specimens can be oriented in a consistent way, it is necessary to have terms for describing embryos (Fig. 2.14). If the egg is approximately spherical with an animal and vegetal pole, then the line joining the two poles is the animal–vegetal axis.

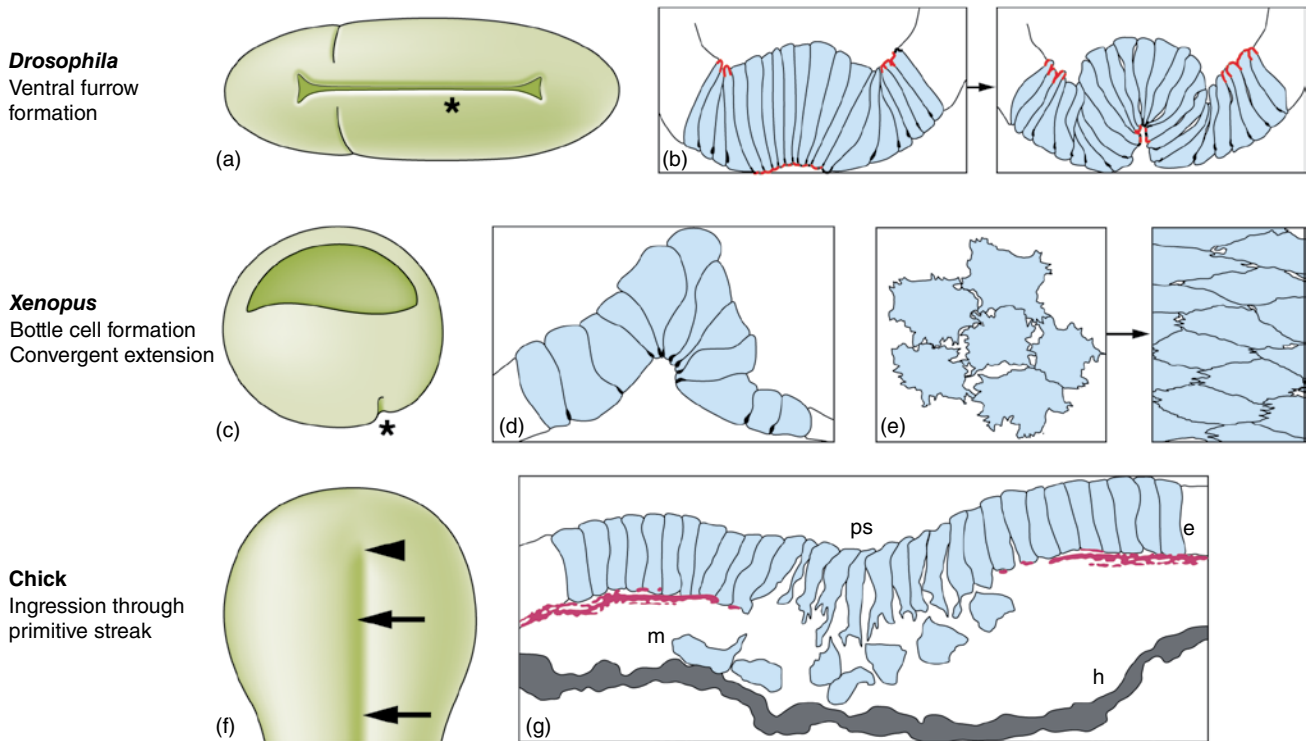

Fig. 2.12 Different processes during gastrulation. (a,b) Ventral furrow formation in *Drosophila*, * indicates the ventral furrow. (c–e) *Xenopus* gastrulation: (c) a bisected embryo, * indicates the dorsal lip; (d) bottle cells appearing at the blastopore; (e) intercalation of cells leading to axial elongation (convergent extension). (f,g) Ingression of cells through the primitive streak of a chick embryo.

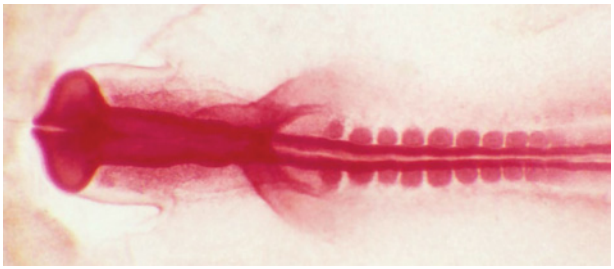

Fig. 2.13 Stage 10 chick embryo, showing the major body structures as cell condensations. Source: Jonathan Slack.

Unfertilized eggs are usually radially symmetrical around this axis, but after fertilization there is often a cytoplasmic rearrangement that breaks the initial radial symmetry and generates a bilateral symmetry. In some organisms, such as *Drosophila*, this may occur earlier, in the oocyte; in others, such as mammals, it may occur later, at a multicellular stage. But even animals such as sea urchins, which are radially symmetrical as adults, or gastropods, which are asymmetrical as adults, still have bilaterally symmetrical early embryos. The change of symmetry means that the animal now has a distinct **dorsal** (upper) and **ventral** (lower) side.

If the animal and vegetal poles are at the top and bottom, then the equatorial plane is the horizontal plane dividing the egg into **animal** and **vegetal** hemispheres, just like the equator of the Earth. Any vertical plane, corresponding to circles of longitude, is called a **meridional** plane. Once the embryo has acquired its bilateral symmetry, then there is a particularly important meridional plane, the **medial** (= **sagittal**) plane, separating the right and left sides of the body. This is often, but not always, the plane of the first cleavage. The **frontal** plane is the meridional plane at right angles to the medial plane, and is often, but not necessarily, the plane of the second cleavage.

Following gastrulation most animals become elongated. The head end is the **anterior**, the tail end is the **posterior**, so the head-to-tail axis is called **anteroposterior** (= craniocaudal or rostrocaudal). The top–bottom axis is called **dorsoventral** and the left–right axes are called **mediolateral**. In human anatomy, because we stand upright on two legs, the term anteroposterior is normally synonymous with dorsoventral, but the term "craniocaudal" remains acceptable for the head-to-tail axis. The terms **proximal** and **distal** are used in relation to appendages, proximal meaning "near the body" and distal meaning "farther away from the body."

Generally, the principal body parts become visible as cell condensations some time after completion of gastrulation. Some phyla, including annelids, arthropods, and chordates, show prominent **segmentation** of the anteroposterior axis. To qualify as segmented an organism should show repeated structures that are similar or identical to each other, are principal rather than minor body parts, and involve contributions from all the germ layers.

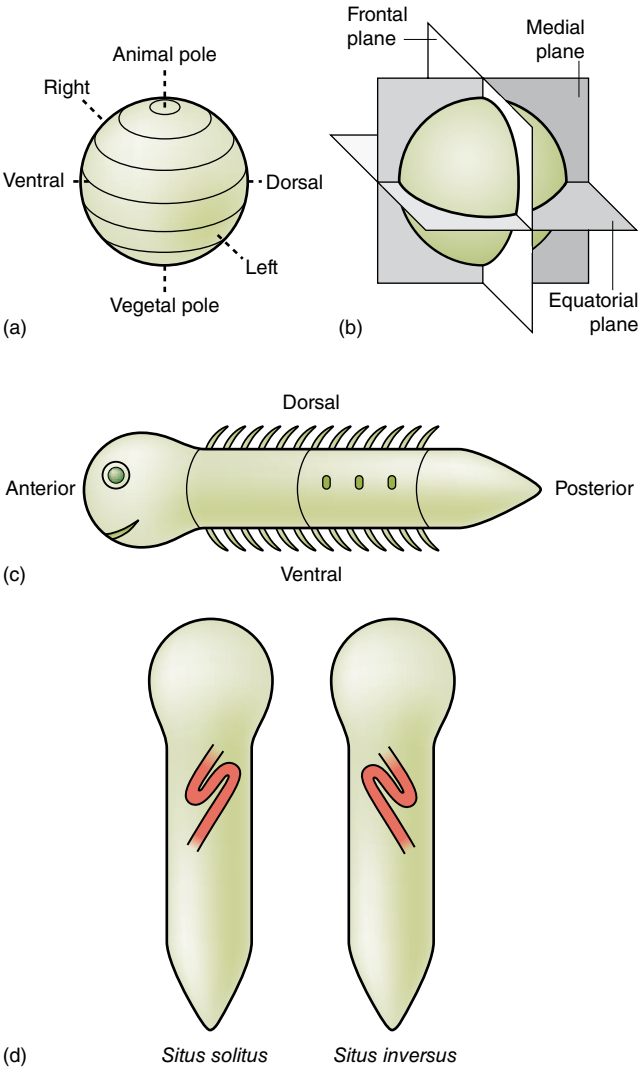

Fig. 2.14 Axes and symmetry. (a) Axes of a fertilized egg after it has acquired dorsoventral asymmetry. (b) Anatomical planes of an early embryo. (c) Principal axes of an animal viewed from the left side. (d) Ventral view of an animal showing deviation from bilateral symmetry.

Although most animals have an overriding bilateral symmetry, this is not exact and there are systematic deviations which make right and left sides slightly different from each other. For example, in mammals the cardiac apex, stomach, and spleen are on the left and the liver, vena cava, and greater lung lobation are on the right. This asymmetrical arrangement is known as ***situs solitus***. If the arrangement is inverted, as occurs in some mutants or experimental situations, it is called ***situs inversus*** (Fig. 2.14d).

Developmental control genes

The state of commitment of different body parts is controlled by the expression of specific sets of developmental control genes.

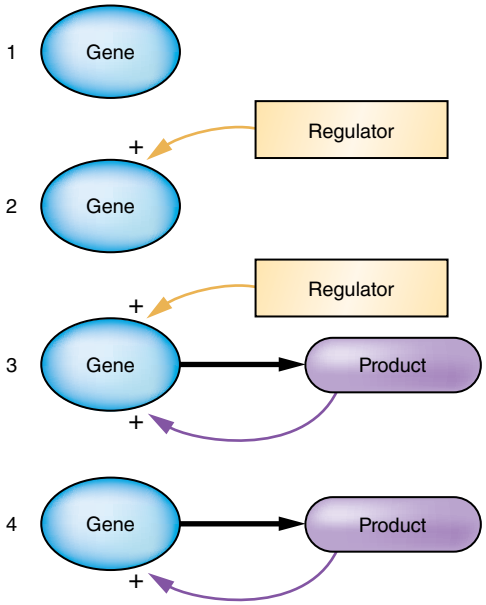

Fig. 2.15 Operation of a bistable switch. The figure depicts a temporal sequence: in step 2 the gene is upregulated by a regulator; in step 3 it is also upregulated by its own product; in step 4 it remains on because of the product, even though the regulator is now gone.

These are sometimes called **homeotic** genes, or **selector** genes. The expression of these genes in embryos is controlled by cytoplasmic determinants or by inducing factors, as explained earlier in this chapter.

Developmental control genes virtually all encode transcription factors, proteins whose function is regulating the activity of other genes. It is important to note that for such genes, as much information is encoded by the "off" state as by the "on" state because the absence of a repressor can be equivalent to the presence of an activator. Although gene expression may occur at any level, for developmental control genes there are often mechanisms that ensure that the steady-state level is either on or off, this being a natural way of ensuring a sharp and discontinuous **threshold** response to a determinant or inductive signal. One way of ensuring that there are just two discrete states of activity for developmental control genes is to have a positive-feedback regulation, as shown in Fig. 2.15. This type of system is called a **bistable switch**, because it has two stable states: on and off. The gene is off when both the regulator and the gene product are absent. It is initially turned on by the regulator, which might be a cytoplasmic determinant or a signal transduction pathway activated by an inducing factor. Once the gene product has accumulated, the gene remains on even if the regulator is removed. This model shows three critically important features of gene regulation in development. Firstly, it can yield a sharp and discontinuous threshold in response to the regulator. Secondly, the system has memory of exposure to the regulator. This is because the gene remains on permanently despite its transient exposure to the regulator. Thirdly, bistable switches are kinetic phenomena.