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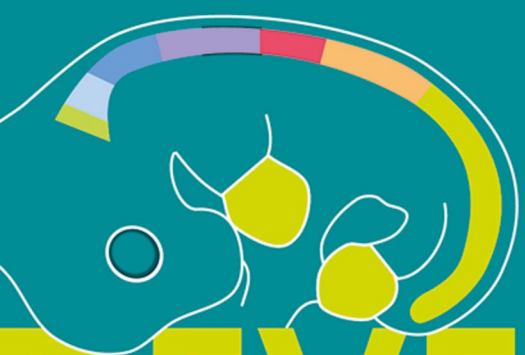

PRINCIPLES OF DEVELOPMENT

SIXTH EDITION

LEWIS WOLPERT
CHERYLL TICKLE
ALFONSO MARTINEZ ARIAS
PETER LAWRENCE | JAMES LOCKE

Principles of Development

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Sixth Edition

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Peter Lawrence

James Locke

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Preface

The fundamental questions about how an embryo develops into a new individual were posed long ago by the ancient Greek scientists and philosophers. Over the centuries, advances in experimental embryology and, more recently, the application of genetics, cell biology, and advances in imaging have revealed many similarities between the development of different organisms, particularly during the early stages, suggesting the existence of general principles underlying the process. Developmental biology is now a mature discipline, and this is the foundation on which this book, focused on principles, was built. We have not included in this sixth edition every new detail that has emerged since the previous edition, which are many and wonderful, but instead, where appropriate, have provided up-to-date examples to illustrate the general principles.

Principles of Development is designed for undergraduates, and we have tried to make these principles as clear as possible and to provide numerous summaries, in both words and pictures. As we understand the principles better, the book should become shorter, but we have not yet achieved this!

We concentrate on the development of vertebrates and *Drosophila* but include other organisms, such as the nematode, the sea urchin, *Hydra*, planarians, and crustaceans, where they best illustrate a concept. Chapter 1 provides a brief history of embryology and an introduction to some of the main general principles and processes involved. Chapter 2 considers the process of pattern formation in laying down the body plan in *Drosophila* (Chapter 2). This small fly has played, and still plays, a central role in elucidating developmental mechanisms. Chapter 3 describes the embryology and genetics of our vertebrate model organisms—*Xenopus*, zebrafish, chick, and mouse—together with some of the main methods used to study them, with coverage in this edition of new methods of gene editing such as CRISPR-Cas9. Although the fundamental principles of development are still largely illuminated by studies on the embryos of model organisms, there is an emerging focus on human embryonic development, and we have expanded our coverage of the topic to include advances in studying early stages, an outline of the development of the human placenta, and a box on human twinning. The mechanisms involved in pattern formation in the early development of our vertebrate model organisms are considered in Chapters 4 and 5. These chapters are organized as in the previous edition, with the process of laying down the early body plan being first described in its entirety in *Xenopus* (Chapter 4), the vertebrate in which the general principles were discovered. This is followed by comparisons with the process in the zebrafish (Chapter 4), and in chick and mouse (Chapter 5). Chapter 5 also considers how the body plan is completed, which mainly rests on studies in chick and mouse embryos. Chapter 6 focuses on pattern formation in two invertebrate model organisms, the nematode and the sea urchin, with an online section on ascidian development.

Chapters 7 and 8 focus on the fundamental processes of morphogenesis and differentiation, respectively, and have been extensively revised, with particular reference to the role of planar cell polarity and convergent extension in Chapter 7, and the impact of single-cell transcriptomics in dissecting cell-fate decisions, with the blood cell lineages as an example, in Chapter 8. Chapter 8 also includes more extensive coverage of the role of epigenetics in development and cell differentiation, and some new boxes, including one describing organoids—three-dimensional organ cultures that mimic tissues and organs—and their potential clinical uses. Chapter 9 deals with germ cells and

fertilization. Organogenesis (Chapter 10) and the development of the nervous system (Chapter 11) are huge topics, so we have had to be very selective in our coverage, but have included a new box in Chapter 10 on mammary gland development and its relevance to understanding breast cancer. Sections of the chapter on the development of the *Drosophila* tracheal system and the mammalian vascular system have been moved to Chapter 7, and other sections from previous editions have been placed online. Growth and regeneration are considered together in the same chapter (Chapter 12). Chapter 13 deals with plant development. This chapter has been updated and includes a new section on vernalization. The last chapter (Chapter 14) deals with development in relation to evolution and has been reorganized and updated throughout, highlighting the impact of genomics and the many insights it has brought to this aspect of developmental biology. Evolution and development are inextricably linked in ways we are only now beginning to understand, with the convergence of genomics and cell biology on embryos. Together with the new technologies for gene editing, genomics is rapidly expanding the range of species accessible to evolutionary studies.

We have assumed that students have some familiarity with basic cell and molecular biology and genetics, but all key concepts, such as the control of gene activity, are explained in the text. There is an extensive Glossary, which means that the book is self-contained. The illustrations are a special feature and have been carefully designed and chosen to illuminate both experiments and mechanisms. New diagrams and photographs are included throughout the book, together with information about their sources. In providing further reading, our prime concern has been to guide the student to particularly helpful papers and reviews, rather than to give credit to all the scientists who have made major contributions: to those whom we have neglected, we apologize.

The main authors for this new edition are, as for the last edition, Lewis Wolpert, Cheryll Tickle, and Alfonso Martinez Arias. We have bid farewell to co-authors of previous editions, Jim Smith, Elliot Meyerowitz, Elizabeth Robertson, and Andrew Lumsden, and thank them for their pivotal contributions over several editions. The remaining long-standing co-author, Peter Lawrence, has been joined by James Locke for this edition, who has revised the chapter on plants. Each chapter has also been reviewed by a number of experts (see page xxv), to whom we are very grateful. The authors made the initial revisions, which were then deciphered, edited, and incorporated by our editor, Eleanor Lawrence, who has been helped for this edition by Amanda Tromans. We thank them both. Eleanor's involvement has been absolutely essential in the preparation of this edition and her expertise and influence pervades the book. Her input has also been invaluable in ensuring that the information in the book is readily accessible to students. The new illustrations were brilliantly drawn or adapted by Matthew McClements, who created the illustrations for the first and subsequent editions.

We are indebted to Roseanna Levermore and Jonathan Crowe at Oxford University Press for their help and suggestions throughout the preparation of this new edition.

L. W.

London
October 2018

C. T.

Bath
October 2018

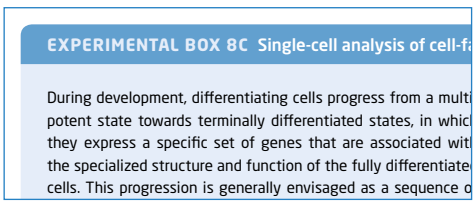
A. M. A.

Cambridge
October 2018

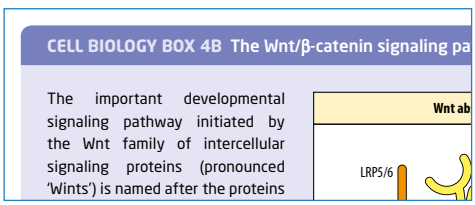
Learning from this book

Principles of Development includes a number of features to help make it easy to use, and to make your learning as effective as possible.

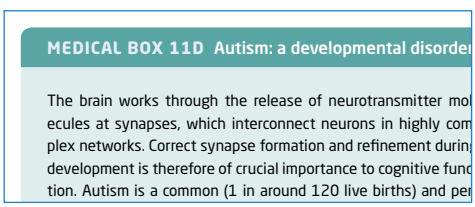
Experimental boxes discuss both classic and current experimental research, demonstrating 'how we know what we know.'



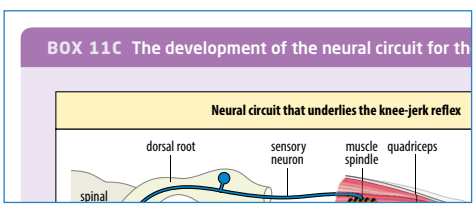
Cell Biology boxes equip you with a robust conceptual framework on which to add further detail from the vast amount of scientific information available to us today.



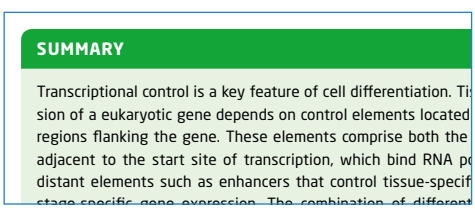
Medical boxes illustrate the direct relevance of developmental biology to medicine and health-related issues.



Special interest boxes highlight topics of interest such as 'The development of the neural circuit for the knee-jerk reflex' in Chapter 11 and the 'Origins of morphological diversity in dogs' in Chapter 14.



Summary boxes provide a brief overview of each main section, which we hope you will find particularly useful when revising for examinations. Summary boxes are augmented by a bullet-point review of the chapter's major concepts at the end of each chapter.



Online resources

The online resources that accompany this text contain additional teaching and learning resources for both lecturers and students.
Visit the site at **www.oup.com/uk/wolpert6e**

For students

Flashcard glossary

Flashcards, which can be downloaded your mobile phone, help you to test your recall of key terminology.

Multiple-choice questions

Use the extensive bank of multiple-choice questions to check your understanding of concepts introduced in the book, and get instant feedback on your progress.

Answer guidance

The authors have written answer guidance to the long-answer questions found at the end of each chapter, so you can check that you have considered all the appropriate points when responding to each question.

Web links and web activities

Links to websites, with notes to explain how each site relates to concepts featured in the book, are provided to help you explore topics in the book in more detail. Complete the associated activities to get to grips with the material in a hands-on way.

In silico practicals

In the textbook, we set out the current understanding of developmental processes and provide some examples of 'how we know what we know' in experimental boxes. However, it is impossible to present the raw data that provide the evidence on which our knowledge is based. The purpose of the practicals is to give you the opportunity to examine raw data and appreciate the way in which results are interpreted and lead to advances in understanding. The *in silico* practicals include questions to help you think more deeply about the material you have learned.

Movies from real research

The movies show key developmental processes occurring in real embryos, to help you visualize the processes of developmental biology as they unfold in three dimensions.

Signaling pathway animations

Custom-made animations of key signaling pathways break down these complex processes into stages, making them easier to understand and remember.

Online extracts

Online extracts provide additional information on a range of extra topics, including:

- P-element-mediated transformation
- Genetic mosaics and mitotic recombination
- Kidney development
- Development of the *Drosophila* compound eye
- Reaction–diffusion mechanisms (extended version)
- How the bird wing evolved
- Ascidians
- Directed dilation
- Chromosome capture techniques

For registered adopters

Electronic artwork

Figures from the book are available to download, for use in lecture slides.

Journal clubs

Journal clubs consist of discussion questions focused around primary literature articles that relate to topics featured in the book. Use these as an additional learning tool to help your students become more adept at assimilating knowledge from the research literature.

Test bank

A test bank of questions is available for you to use when assessing your students.

About the authors

Lewis Wolpert is Emeritus Professor of Biology as Applied to Medicine, in the Department of Anatomy and Developmental Biology, University College London, London, UK. He is the author of *The Triumph of the Embryo, A Passion for Science, The Unnatural Nature of Science,* and *Six Impossible Things Before Breakfast*.

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Matthew McClements is an illustrator who specializes in design for scientific, technical, and medical communication.

Summary of contents

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Reviewer acknowledgments

We would like to reiterate our thanks to the people who contributed to the first five editions. Many people gave their advice based on the fifth edition, and others reviewed the draft chapters for the sixth edition as they emerged. We wish to express our gratitude to the following colleagues.

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1

History and basic concepts

- The origins of developmental biology
- A conceptual tool kit

The aim of this chapter is to provide a conceptual framework for the study of development. We start with a brief history of the study of embryonic development, which illustrates how some of the key questions in developmental biology were first formulated, and continue with some of the essential principles of development. The big question is: How does a single cell—the fertilized egg—give rise to a multicellular organism, in which a multiplicity of different cell types are organized into tissues and organs to make up a three-dimensional body? This question can be studied from many different viewpoints, all of which have to be fitted together to obtain a complete picture of development: which genes are expressed, and when and where; how cells communicate with each other; how a cell's developmental fate is determined; how cells proliferate and differentiate into specialized cell types; and how major changes in body shape are produced. All the information for embryonic development is contained within the fertilized egg. We shall see that an organism's development is ultimately driven by the regulated expression of its genes in space and time, determining which proteins are present in which cells and when. In turn, proteins largely determine how a cell behaves. The genes provide a generative program for development, not a blueprint, as their actions are translated into developmental outcomes through cellular behavior such as intercellular signaling, cell proliferation, cell differentiation, and cell movement.

The development of a multicellular organism from a single cell—the fertilized egg—is a brilliant triumph of evolution. The fertilized egg divides to give rise to many millions of cells, which form structures as complex and varied as eyes, arms, heart, and brain. This amazing achievement raises a multitude of questions. How do the cells arising from division of the fertilized egg become different from each other? How do they become organized into structures such as limbs and brains? What controls the behavior of individual cells so that such highly organized patterns emerge? How are the organizing principles of development embedded within the egg, and, in particular, within the genetic material, DNA? Much of the excitement in developmental biology today comes from our growing understanding of how genes direct these developmental processes, and genetic control is one of the main themes of this book. Thousands of genes are involved in controlling development, but we will focus only on those that have key roles, and illustrate general principles.

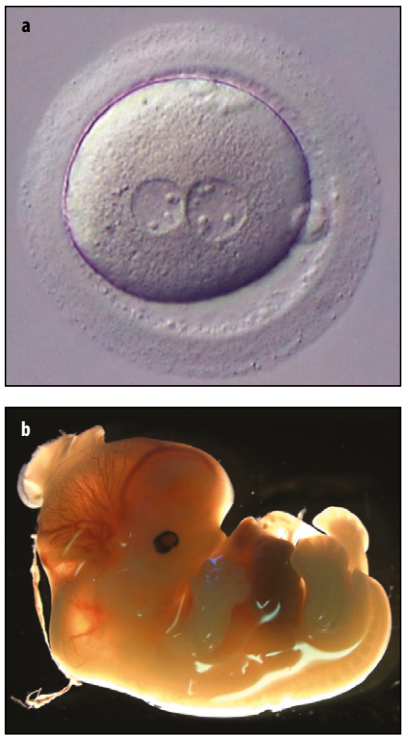

Fig. 1.1 Human fertilized egg and embryo. (a) Human fertilized egg. The nuclear membranes of the sperm and egg nuclei (pronuclei) have not yet broken down to allow the parental chromosomes to mingle. (b) Human embryo at around 51 days' gestation (Carnegie stage 20), which is equivalent to a mouse embryo at 13.5 days post-fertilization. A human embryo at this stage is about 21–23 mm long.

(a) Courtesy of A. Doshi, CRGH, London. (b) Reproduced courtesy of the MRC/ Wellcome-funded Human Developmental Biology Resource.

Fig. 1.2 The South African claw-toed frog, *Xenopus laevis*. Scale bar = 1 cm.

Photograph courtesy of J. Smith.

Understanding how embryos develop is a huge intellectual challenge, and one of the ultimate aims of the science of **developmental biology** is to understand how we humans develop (Fig. 1.1). We need to understand human development for several reasons. We need to properly understand why it sometimes goes wrong and why a **fetus** may fail to be born or a baby be born with congenital abnormalities. The link here with genetic control of development is very close, as mutations in genes can lead to abnormal development; environmental factors, such as drugs and infections, can affect it too. Another area of medical research related to developmental biology is regenerative medicine—finding out how to use cells to repair damaged tissues and organs. The focus of regenerative medicine is currently on **stem cells**. Stem cells that can proliferate and give rise to all the different tissues of the body are present in embryos. These, and the stem cells with more limited developmental potential that are found in adult tissues, are discussed in Chapter 8. Cancer cells also display some properties of embryonic cells, such as the ability to divide indefinitely, and so the study of embryonic cells and their behaviour could lead to new and better treatments for cancer, as many of the same genes are involved.

The development of an embryo from the fertilized egg is known as **embryogenesis**. One of the first tasks that cells have in an embryo is to lay down the overall body plan of the organism, and we shall see that different organisms solve this fundamental problem in several ways. The focus of this book is mainly on animal development, in particular that of vertebrates—frogs, birds, fish, and mammals—whose early development is discussed in Chapters 3–5. We also look at selected invertebrates, particularly the fruit fly and the nematode worm, and also the sea urchin. Our understanding of the genetic control of development is founded on work with fruit flies and nematodes, where it is also most advanced, and the main features of their early development are considered in Chapters 2 and 6, respectively. The fruit fly is also used throughout the book to illustrate particular aspects of development. In Chapter 13 we look briefly at some aspects of plant development, which differs in many respects from that of animals but involves similar basic principles.

Morphogenesis, or the development of form, is discussed in Chapter 7. In Chapter 9 we look at how sex is determined and how germ cells develop. The differentiation of unspecialized cells into cells that carry out particular functions, such as muscle cells and blood cells, is considered in Chapter 8. Structures such as the vertebrate limb, and organs such as insect and vertebrate eyes, the heart and the nervous system, illustrate the problems of multicellular organization and tissue differentiation in embryogenesis, and we consider some of these systems in detail in Chapters 10 and 11. The study of developmental biology, however, goes well beyond the development of the embryo. Post-embryonic growth and aging, how some animals undergo metamorphosis, and how animals can regenerate lost organs is discussed in Chapter 12. Taking a longer view, we shall consider in Chapter 14 how developmental mechanisms have evolved and how they constrain the very process of evolution itself.

One might ask whether it is necessary to cover so many different organisms in order to understand the basic features of development. The answer is yes. Developmental biologists do, indeed, believe that there are general principles of development that apply to all animals, but life is too wonderfully diverse to find all the answers in a single organism. As it is, developmental biologists have tended to focus their efforts on a relatively small number of animals, chosen because they were convenient to study and amenable to experimental manipulation or genetic analysis. This is why some creatures, such as the frog *Xenopus laevis* (Fig. 1.2) and the fruit fly *Drosophila melanogaster*, have such a dominant place in developmental biology. Similarly, work with the thale cress, *Arabidopsis thaliana*, has uncovered many features of plant development.

One of the most exciting and satisfying aspects of developmental biology is that understanding a developmental process in one organism can help to illuminate similar processes elsewhere—for example, giving insights into how humans develop. Nothing illustrates this more dramatically than the influence that our understanding of *Drosophila* development, and especially of its genetic basis, has had throughout

developmental biology. The identification of genes controlling early embryogenesis in *Drosophila* has led to the discovery of related genes being used in similar ways in the development of mammals and other vertebrates. Such discoveries encourage us to believe in the existence of general developmental principles.

Amphibians have long been favorite organisms for studying early development because their eggs are large and their embryos are easy to grow in a simple culture medium and relatively easy to experiment on. Embryogenesis in the South African frog *Xenopus* (Box 1A) illustrates some of the basic stages of development in all animals.

In the rest of this chapter we first look briefly at the history of **embryology**—as the study of developmental biology used to be called. The term developmental biology itself is of much more recent origin and reflects the appreciation that development is not restricted to the embryo alone. Traditionally, embryology described experimental results in terms of morphology and cell fate, but we now understand development in terms of molecular genetics and cell biology as well. In the second part of the chapter we will introduce some key concepts that are used over and over again in studying and understanding development.

The origins of developmental biology

Many questions in embryology were first posed hundreds, and in some cases thousands, of years ago. Appreciating the history of these ideas helps us to understand why we approach developmental problems in the way that we do today.

1.1 Aristotle first defined the problem of epigenesis versus preformation

A scientific approach to explaining development started with Hippocrates in Greece in the fifth century BC. Using the ideas current at the time, he tried to explain development in terms of the principles of heat, wetness, and solidification. About a century later the study of embryology advanced when the Greek philosopher Aristotle formulated a question that was to dominate much thinking about development until the end of the nineteenth century. Aristotle addressed the problem of how the different parts of the embryo were formed. He considered two possibilities: one was that everything in the embryo was preformed from the very beginning and simply got bigger during development; the other was that new structures arose progressively, a process he termed **epigenesis** (which means 'upon formation') and that he likened metaphorically to the 'knitting of a net'. Aristotle favored epigenesis and his conjecture was correct. Aristotle's influence on European thought was enormous and his ideas remained dominant well into the seventeenth century. The contrary view to epigenesis, namely that the embryo was preformed from the beginning, was championed anew in the late seventeenth century. Many could not believe that physical or chemical forces could mold a living entity such as the embryo. Along with the contemporaneous background of belief in the divine creation of the world and all living things, was the belief that all embryos had existed from the beginning of the world, and that the first embryo of a species must contain all future embryos.

Even the brilliant seventeenth-century Italian embryologist Marcello Malpighi could not free himself from preformationist ideas. While he provided a remarkably accurate description of the development of the chick embryo, he remained convinced, against the evidence of his own observations, that the fully formed embryo was present from the beginning (Fig. 1.3). He argued that at very early stages the parts were so small that they could not be seen, even with his best microscope. Other preformationists believed that the sperm contained the embryo, and some even claimed to see a tiny human—an homunculus—in the head of each human sperm (Fig. 1.4).

The preformation/epigenesis issue was vigorously debated throughout the eighteenth century. But the problem could not be resolved until one of the great advances in biology had taken place—the recognition that living things, including embryos, were composed of cells.

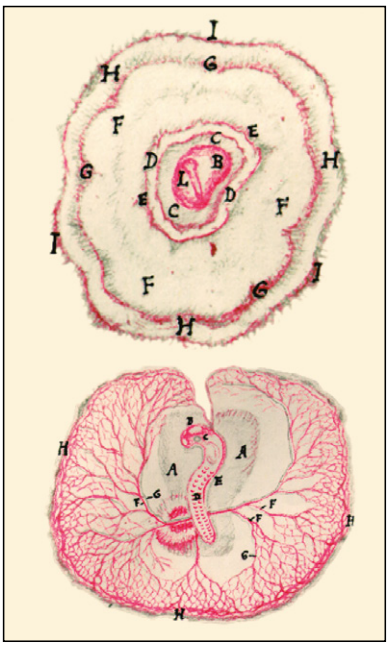

Fig. 1.3 Malpighi's description of the chick embryo. The figure shows Malpighi's drawings, made in 1673, depicting the early embryo (top) and at 2 days' incubation (bottom). His drawings accurately illustrate the shape and blood supply of the embryo. *Reprinted by permission of the President and Council of the Royal Society.*

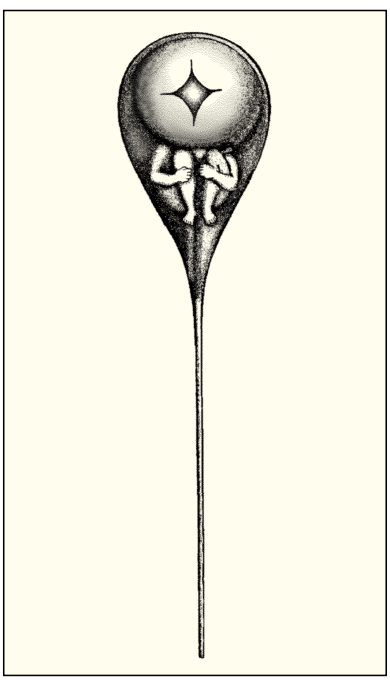

Fig. 1.4 Some preformationists believed that an homunculus was curled up in the head of each sperm. *An imaginative drawing, after N. Harspeler (1694).*

BOX 1A Basic stages of *Xenopus laevis* development

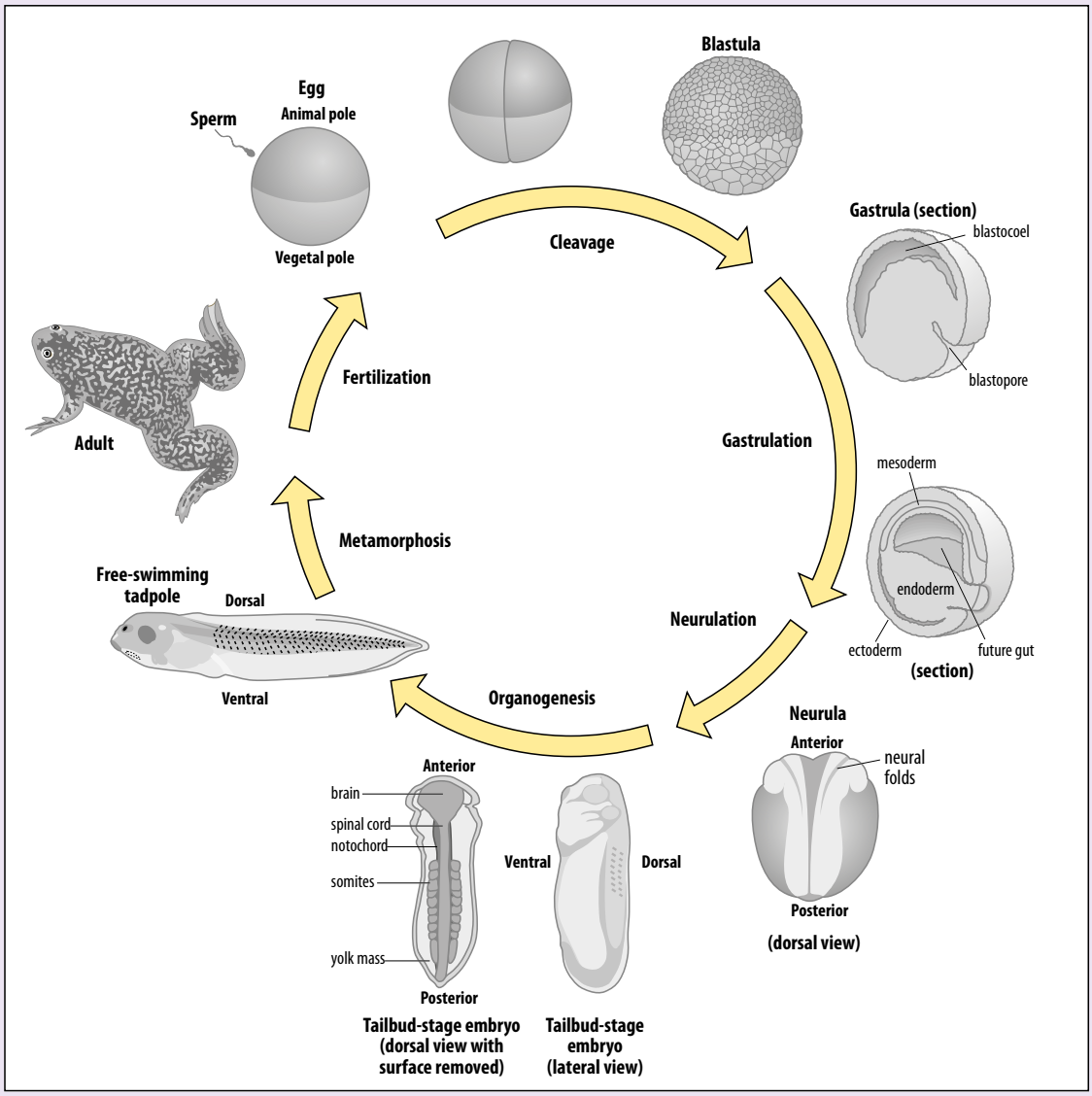

Fig. 1

1.2 Cell theory changed how people thought about embryonic development and heredity

The invention of the microscope around 1600 was essential for the discovery of cells, but the 'cell theory' of life was only developed between 1820 and 1880 by, among others, the German botanist, Matthias Schleiden, and the physiologist, Theodor Schwann. It recognized that all living organisms consist of cells, that these are the basic units of life, and that new cells can only be formed by the division of pre-existing cells. The cell theory was one of the most illuminating advances in biology, and had an enormous impact. Multicellular organisms, such as animals and plants, could now be viewed as communities of cells. Development could not, therefore, be based on preformation, but must be by epigenesis, because during development many

Although vertebrate development is varied, there are a number of basic stages that can be illustrated by following the development of the frog *Xenopus laevis* (Fig. 1). The unfertilized egg is a large cell. It has a pigmented upper surface (the **animal pole**) and a lower region (the **vegetal pole**) characterized by an accumulation of yolk granules.

After **fertilization** of the egg by a **sperm**, and the fusion of male and female pronuclei, **cleavage** begins. Cleavages are mitotic divisions in which cells do not grow between each division, and so with successive cleavages the cells become smaller. After about 12 division cycles, the embryo, now known as a **blastula**, consists of many small cells surrounding a fluid-filled cavity (the **blastocoel**) above the larger yolky cells. Already, changes have occurred within the cells and they have interacted with each other so that the three **germ layers**—**mesoderm**, **endoderm**, and **ectoderm**—are specified (see Box 1C). The animal region gives rise to ectoderm, which forms both the epidermis of the skin and the nervous system. The vegetal region gives rise to the future endoderm and mesoderm, which are destined to form internal organs. At this stage, these cells are still on the surface of the embryo. During the next stage—**gastrulation**—there is a dramatic rearrangement of cells; the endoderm and mesoderm move inside, and the basic body plan of the tadpole is established. Internally, the mesoderm gives rise to a rod-like structure (the notochord), which runs from the head to the tail, and lies centrally beneath the future nervous system. On either side of the notochord are segmented blocks of mesoderm called somites, which will give rise to the muscles and vertebral column, as well as the dermis of the skin (somites can be seen in the cutaway view of the later tailbud-stage embryo).

Shortly after gastrulation, the ectoderm above the notochord folds to form a tube (the **neural tube**), which gives rise to the brain and spinal cord—a process known as **neurulation**. By this time, other organs, such as limbs, eyes, and gills, are specified at their future locations, but only develop a little later, during **organogenesis**. During organogenesis, specialized cells such as muscle, cartilage, and neurons differentiate. By four days after fertilization, the embryo has become a free-swimming tadpole with typical vertebrate features.

new cells are generated by division from the egg, and new types of cells are formed. A crucial step forward in understanding development was the recognition, in the 1840s, that the egg itself is but a single, albeit specialized, cell.

An important advance in embryology was the proposal by the nineteenth-century German biologist, August Weismann, that an offspring does not inherit its characteristics from the body (the soma) of the parent but only from the **germ cells**—egg and sperm. Weismann drew a fundamental distinction between germ cells and the body cells or **somatic cells** (Fig. 1.5). Characteristics acquired by the body during an animal's life cannot be transmitted to the germline. As far as heredity is concerned, the body is merely a carrier of germ cells. As the English novelist and essayist Samuel Butler put it: 'A hen is only an egg's way of making another egg.'

Work on sea urchin eggs showed that after fertilization the egg contains two nuclei, which eventually fuse; one of these nuclei belongs to the egg, whereas the other comes from the sperm. Fertilization therefore results in a single cell—the **zygote**—carrying a nucleus with contributions from both parents, and it was concluded that the cell nucleus must contain the physical basis of heredity. The climax of this line of research was the

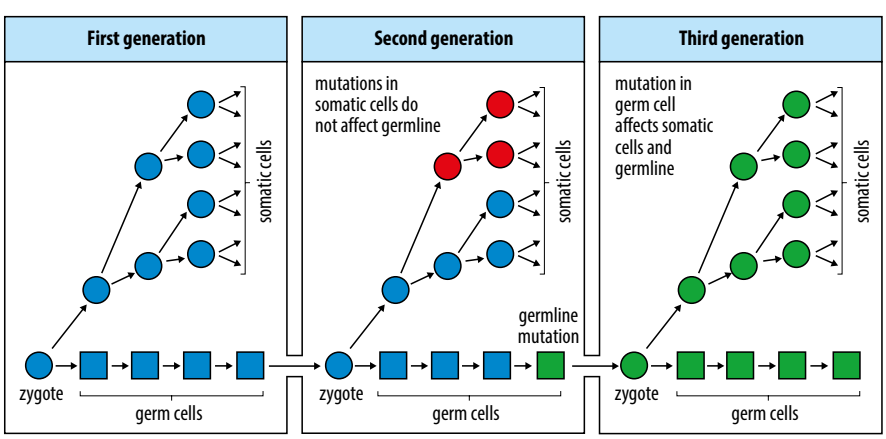

Fig. 1.5 The distinction between germ cells and somatic cells. In each generation a germ cell contributes to the zygote, which gives rise to both somatic cells and germ cells, but inheritance is through the germ cells only (first panel). Changes that occur due to a mutation (red) in a somatic cell can be passed on to its daughter cells but do not affect the germline, as shown in the second panel. In contrast, a mutation in the germline (green) in the second generation will be present in every cell in the body of the new organism to which that cell contributes, and will also be passed on to the third and future generations through the germline, as shown in the third panel.

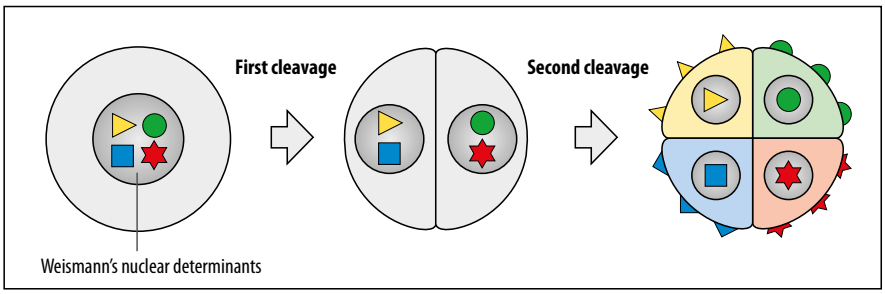

Fig. 1.6 Weismann's theory of nuclear determination. Weismann assumed that there were factors in the nucleus that were distributed asymmetrically to daughter cells during cleavage and directed their future development.

demonstration, towards the end of the nineteenth century, that the chromosomes within the nucleus of the zygote are derived in equal numbers from the two parental nuclei, and the recognition that this provided a physical basis for the transmission of genetic characters according to the laws developed by the Austrian botanist and monk, Gregor Mendel. The number of chromosomes is kept constant from generation to generation by a specialized type of cell division that produces the germ cells, called **meiosis**, which halves the chromosome number; the full complement of chromosomes is then restored at fertilization. The zygote and the somatic cells that arise from it divide by the process of **mitosis**, which maintains chromosome number (Box 1B). Germ cells contain a single copy of each chromosome and are called **haploid**, whereas germ-cell precursor cells and the other somatic cells of the body contain two copies and are called **diploid**.

1.3 Two main types of development were originally proposed

The next big question was how cells became different from one another during embryonic development. With the increasing emphasis on the role of the nucleus, in the 1880s Weismann put forward a model of development in which the nucleus of the zygote contained a number of special factors, or **determinants** (Fig. 1.6). He proposed that while the fertilized egg underwent the rapid cycles of cell division known as **cleavage** (see Box 1A), these nuclear determinants would be distributed unequally to the daughter cells and so would control the cells' future development. The fate of each cell was therefore predetermined in the egg by the factors it would receive during cleavage. This type of model was termed 'mosaic', as the egg could be considered to be a mosaic of discrete localized determinants. Central to Weismann's theory was the assumption that early cell divisions must make the daughter cells quite different from each other as a result of unequal distribution of nuclear components.

In the late 1880s, initial support for Weismann's ideas came from experiments carried out independently by the German embryologist, Wilhelm Roux, who experimented with frog embryos. Having allowed the first cleavage of a fertilized frog egg, Roux destroyed one of the two cells with a hot needle and found that the remaining cell developed into a well-formed half-larva (Fig. 1.7). He concluded that the

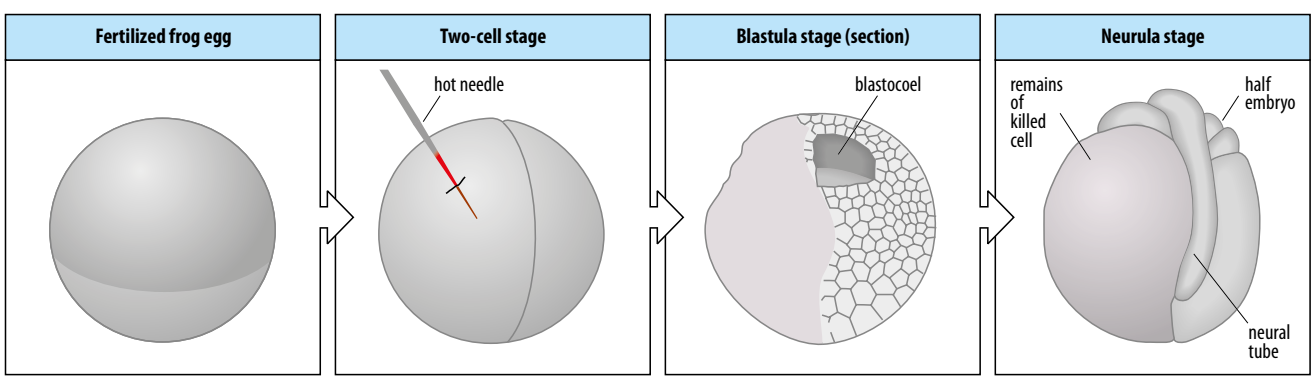

Fig. 1.7 Roux's experiment to investigate Weismann's theory of mosaic development. After the first cleavage of a frog embryo, one of the two cells is killed by pricking it with a hot needle; the other remains undamaged. At the blastula stage the undamaged cell can be seen to have divided as normal into many cells that fill half of the embryo. The development of the blastocoel, a small fluid-filled space in the center of the blastula, is also restricted to the undamaged half. In the damaged half of the embryo, no cells appear to have formed. At the neurula stage, the undamaged cell has developed into something resembling half a normal embryo.

CELL BIOLOGY BOX 1B The mitotic cell cycle

When a eukaryotic cell duplicates itself it goes through a fixed sequence of events called the **cell cycle**. The cell grows in size, the DNA is replicated, and the replicated chromosomes then undergo mitosis and become segregated into two daughter nuclei. Only then can the cell divide to form two daughter cells, which can go through the whole sequence again.

The standard eukaryotic mitotic cell cycle is divided into well-marked phases (Fig. 1). At the M phase, mitosis and cell cleavage give rise to two new cells. The rest of the cell cycle, between one M phase and the next, is called interphase. Replication of DNA occurs during a defined period in interphase, the S phase (the S stands for synthesis of DNA). Preceding S phase is a period known as G_1 (the G stands for gap), and after it another interval known as G_2, after which the cells enter mitosis (see Fig. 1). G_1, S phase, and G_2 collectively make up interphase, the part of the cell cycle during which cells synthesize proteins and grow, as well as replicate their DNA. When somatic cells are not proliferating they are usually in a state known as G_0, into which they withdraw after mitosis. The decision to enter G_0 or to proceed through G_1 may be controlled by both intracellular state and extracellular signals such as growth factors. Growth factors enable the cell to proceed out of G_0 and progress through the cell cycle. Cells such as neurons and skeletal muscle cells, which do not divide after differentiation, are permanently in G_0.

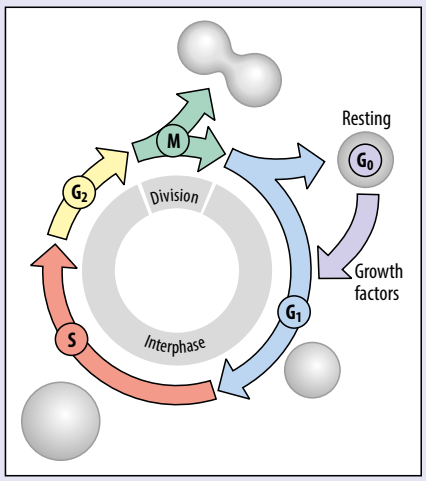

Fig. 1

Particular phases of the cell cycle are absent in some cells: during cleavage of the fertilized *Xenopus* egg G_1 and G_2 are virtually absent, and cells get smaller at each division. In *Drosophila* salivary glands there is no M phase, as the DNA replicates repeatedly without mitosis or cell division, leading to the formation of giant **polytene chromosomes**.

'development of the frog is based on a mosaic mechanism, the cells having their character and fate determined at each cleavage'.

But when Roux's fellow countryman, Hans Driesch, repeated the experiment on sea urchin eggs, he obtained quite a different result (Fig. 1.8). He wrote later: 'But things turned out as they were bound to do and not as I expected; there was, typically, a

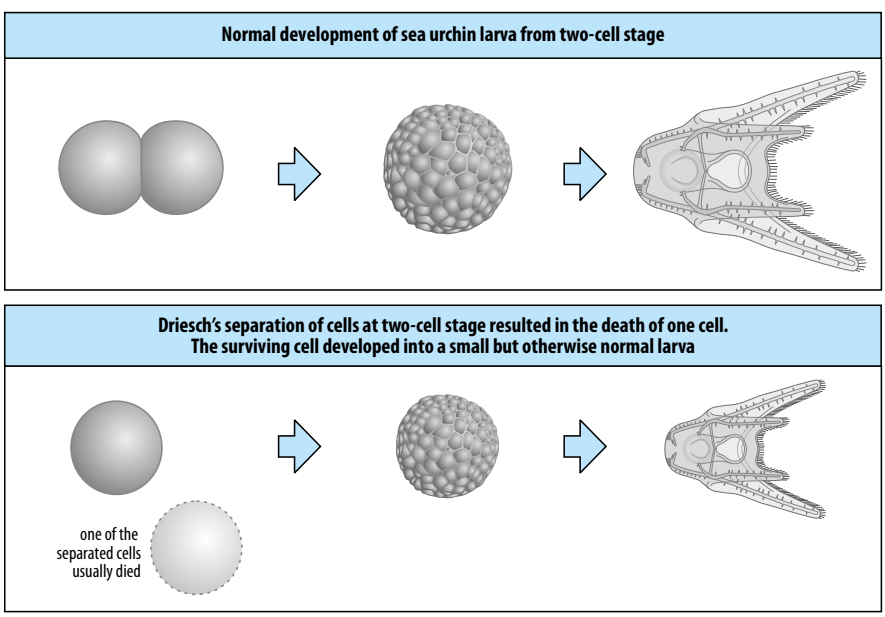

Fig. 1.8 The outcome of Driesch's experiment on sea urchin embryos, which first demonstrated the phenomenon of regulation. After separation of cells at the two-cell stage, the remaining cell developed into a small, but whole, normal larva. This is the opposite of Roux's earlier finding that when one of the cells of a two-cell frog embryo is damaged, the remaining cell develops into a half-embryo only (see Fig. 1.7).

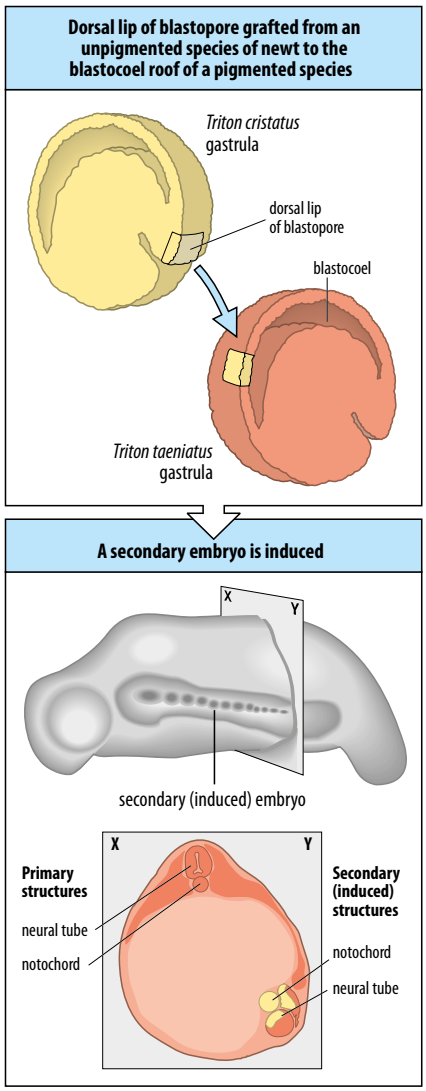

Fig. 1.9 The dramatic demonstration by Spemann and Mangold of the induction of a new main body axis by the organizer region in the early amphibian gastrula. A piece of tissue (yellow) from the dorsal lip of the blastopore of a newt (*Triton cristatus*) gastrula is grafted to the opposite side of a gastrula of another, pigmented, newt species (*Triton taeniatus*, pink). The grafted tissue induces a new body axis containing neural tube and somites. The unpigmented graft tissue forms a notochord at its new site (see section in lower panel), but most of the neural tube and the other structures of the new axis have been induced from the pigmented host tissue. The organizer region discovered by Spemann and Mangold is known as the Spemann organizer.

whole gastrula on my dish the next morning, differing only by its small size from a normal one; and this small but whole gastrula developed into a whole and typical larva.'

Driesch had completely separated the cells at the two-cell stage and obtained a normal but small larva. That was just the opposite of Roux's result, and was the first clear demonstration of the developmental process known as regulation. The experiment of Roux on frogs was later repeated by the American T.H. Morgan, who separated the two blastomeres instead of killing one of them and leaving it attached, and he obtained the same result as Driesch did with sea urchins. This showed the general ability of vertebrate embryos to regulate, that is, to restore normal development, even if some portions are removed or rearranged very early in development. The basis for this phenomenon is explained later in the chapter. The extent to which embryos can regulate differs in different species and we shall see many examples of regulation throughout the book. The existence of regulation does not mean, however, that the unequal distribution of determinants that make two daughter cells different from each other is not important during development. But Weismann was wrong in one crucial respect, in that such determinants are not nuclear, but are located in the cell cytoplasm. We shall see many examples of developmentally important proteins and RNAs that act in this way as **cytoplasmic determinants**.

1.4 The discovery of induction showed that one group of cells could determine the development of neighboring cells

The fact that embryos can regulate implies that cells must communicate and interact with each other, but the central importance of **cell–cell interactions** in embryonic development was not really established until the discovery of the phenomenon of **induction**. This is where one cell, or tissue, directs the development of another, neighboring, cell or tissue.

The importance of induction and other cell–cell interactions in development was proved dramatically in 1924 when Hans Spemann and his assistant, Hilde Mangold, carried out a now famous transplantation experiment in amphibian embryos. They showed that a partial second embryo could be induced by grafting one small region of an early newt embryo onto another at the same stage (Fig. 1.9). The grafted tissue was taken from the dorsal lip of the **blastopore**—the slit-like invagination that forms where gastrulation begins on the dorsal surface of the amphibian embryo (see Box 1A). This small region they called the **organizer**, as it seemed to be ultimately responsible for controlling the organization of a complete embryonic body; it is now known as the **Spemann–Mangold organizer**, or just the **Spemann organizer**. For their discovery, Spemann received the Nobel Prize for Physiology or Medicine in 1935, the first Nobel Prize ever given for embryological research. Sadly, Hilde Mangold had died earlier, in an accident, and so could not be honored.

1.5 Developmental biology emerged from the coming together of genetics and embryology

When Mendel's laws were rediscovered in 1900 there was a great surge of interest in mechanisms of inheritance, particularly in relation to evolution, but less so in relation to embryology. Genetics was seen as the study of the transmission of hereditary elements from generation to generation, whereas embryology was the study of how an individual organism develops and, in particular, how cells in the early embryo became different from each other. Genetics seemed, in this respect, to be irrelevant to development.

The fledgling science of genetics was put on a firm conceptual and experimental footing in the first quarter of the twentieth century by T.H. Morgan. Morgan chose the fruit fly *D. melanogaster* as his experimental organism. He noticed a fly with white eyes rather than the usual red eyes, and by careful cross-breeding he showed that inheritance of this mutant trait was linked to the sex of the fly. He found three other sex-linked traits and worked out that they were each determined by three distinct 'genetic loci', which occupied different positions on the same chromosome, the fly's

X chromosome. The rather abstract hereditary 'factors' of Mendel had been given reality. But even though Morgan was originally an embryologist, he made little headway in explaining development in terms of genetics. That had to wait until the nature of the gene was better understood.

An important concept in understanding how genes influence physical and physiological traits is the distinction between **genotype** and **phenotype**. This was first put forward by the Danish botanist, Wilhelm Johannsen, in 1909. The genetic endowment of an organism—the genetic information it inherits from its parents—is the genotype. The organism's visible appearance, internal structure, and biochemistry comprise the phenotype. While the genotype certainly controls development, environmental factors interacting with the genotype influence the phenotype. Despite having identical genotypes, identical twins can develop differences in their phenotypes as they grow up (Fig. 1.10), and these tend to become more evident with age.

Following Morgan's discoveries in genetics, the problem of development could now be posed in terms of the relationship between genotype and phenotype: how the genetic endowment becomes 'translated' or 'expressed' during development to give rise to a functioning organism. But the coming together of genetics and embryology was slow and tortuous. The discovery in the 1940s that genes are made of DNA and encode proteins was a major turning point. It was already clear that the properties of a cell are determined by the proteins it contains, and so the fundamental role of genes in development could at last be appreciated. By controlling which proteins were made in a cell, genes could control the changes in cell properties and behaviour that occurred during development. A further major advance in the 1960s was the discovery that some genes encode proteins that control the activity of other genes.

Fig. 1.10 The difference between genotype and phenotype. These identical twins have the same genotype because one fertilized egg split into two during development. Their slight difference in appearance is due to non-genetic factors, such as environmental influences.

Photograph courtesy of Robert and Lewis McCaffrey.

1.6 Development is studied mainly through selected model organisms

Although the embryology of many different species has been studied at one time or another, a relatively small number of organisms provide most of our knowledge about developmental mechanisms. We can thus regard them as 'models' for understanding the processes involved, and they are often called **model organisms**. Sea urchins and amphibians were the main animals used for the first experimental investigations because their developing embryos are easy to obtain and, in the case of amphibians, relatively easy to manipulate experimentally, even at quite late stages. Among vertebrates, the frog *Xenopus laevis*, the mouse (*Mus musculus*), the chicken (*Gallus gallus*), and the zebrafish (*Danio rerio*) are the main model organisms now studied. Among invertebrates, the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* have been the focus of most attention, because a great deal is known about their developmental genetics and they can be easily genetically modified. Two Nobel prizes have been awarded for discoveries about development in *Drosophila* and *Caenorhabditis*, respectively. With the advent of modern methods of genetic analysis, there has also been a resurgence of interest in the sea urchin *Strongylocentrotus purpuratus.* For plant developmental biology, *Arabidopsis thaliana* serves as the main model organism. The life cycles and background details for these model organisms are given in the relevant chapters later in the book. The evolutionary relationships of these organisms are shown in Figure 1.11.

The reasons for these choices are partly historical—once a certain amount of research has been done on one animal it is more efficient to continue to study it rather than start at the beginning again with another species—and partly a question of ease of study and biological interest. Each species has its advantages and disadvantages as a developmental model. The chick embryo, for example, has long been studied as a model for vertebrate development because fertile eggs are easily available and the embryo withstands experimental microsurgical manipulation very well. A disadvantage, however, was that until very recently little was known about the chick's developmental genetics. In contrast, we know a great deal about the genetics of the mouse, although the mouse is more difficult to study in some ways, as development