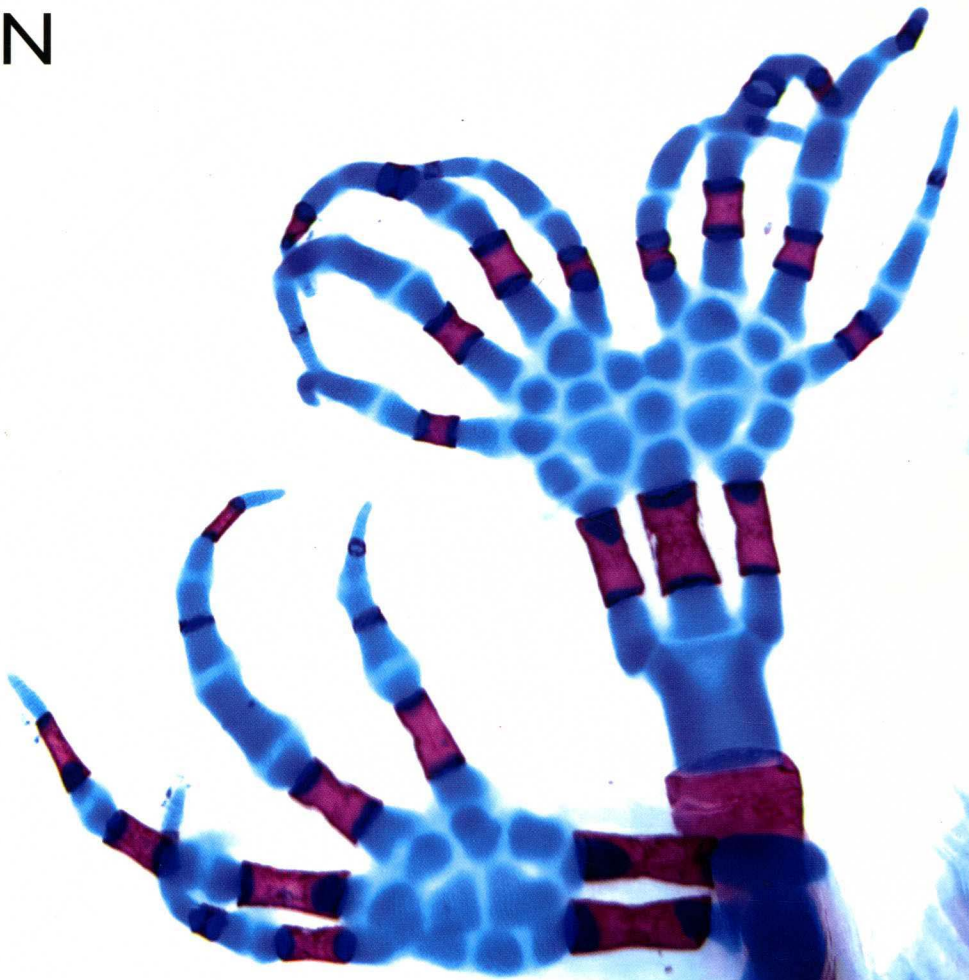
ESSENTIAL DEVELOPMENTAL BIOLOGY

THIRD EDITION



Jonathan M.W. Slack

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3rd Edition

Essential Developmental Biology

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WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication

This edition first published 2013; © 2001, 2006 Blackwell Publishing Ltd, © 2013 by John Wiley & Sons, Ltd

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Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
111 River Street, Hoboken, NJ 07030-5774, USA

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Library of Congress Cataloging-in-Publication Data

Slack, J. M. W. (Jonathan Michael Wyndham), 1949–
Essential developmental biology / Jonathan M.W. Slack. – 3rd ed.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-118-02286-3 (hardback : alk. paper)
ISBN 978-0-470-92351-1 (paperback : alk. paper)
1. Developmental biology–Laboratory manuals. I. Title.
QH491.S6 2012
571.8'6–dc23
2012008542

A catalogue record for this book is available from the British Library.

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Cover design by Design Deluxe

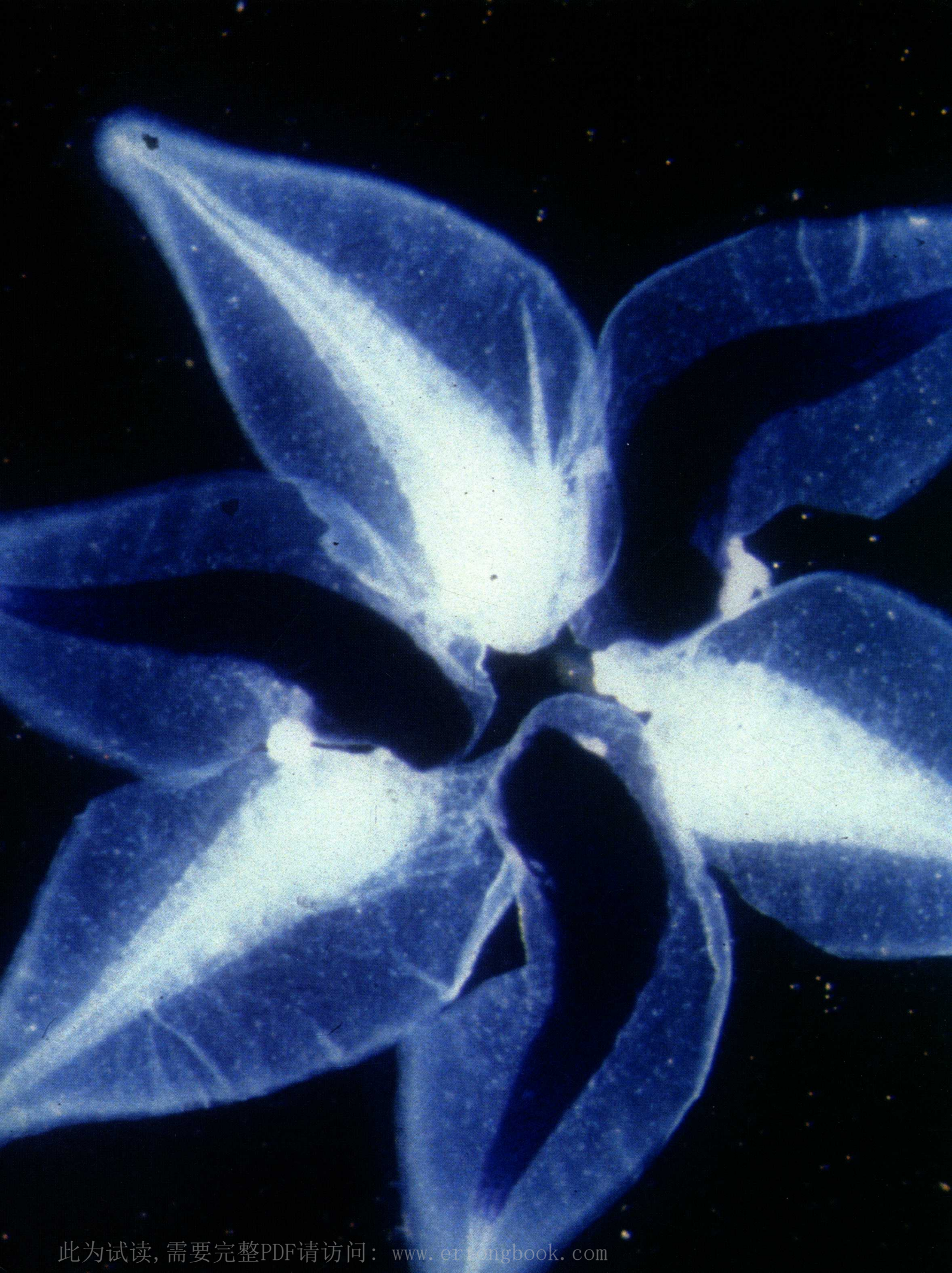
Set in 9.5/12pt Minion by Toppan Best-set Premedia Limited, Hong Kong

Printed and bound in Singapore by Markono Print Media Pte Ltd

1 2013

Cover image: The cover shows a double posterior limb of an axolotl (a type of salamander), together with a normal limb. The double posterior limb arose after grafting a strip of limb rudiment tissue into the flank of a host embryo. This means that a polarizing region develops on both sides, leading to the double posterior symmetry (see Chapter 15). The specimen was made by the author using the technique first described in Slack, JMW. *Determination of polarity in the amphibian limb*, *Nature* 1976: 261:44–46. It was stained and photographed by Drs Ying Chen and Gufa Lin. Red indicates bone and blue indicates cartilage.

Essential Developmental Biology



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 second to the fourth year, and also for first year graduate students. The first and second editions were "road tested" by myself and by many other instructors, and were found 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.

The book is arranged in four sections and the order of topics is intended to represent a logical progression. The first section introduces the basic concepts and techniques. The second covers the six main "model organisms," *Xenopus*, zebrafish, chick, mouse, *Drosophila,* and *Caenorhabditis elegans*, describing their early development to the stage of the general body plan. The third deals with organ development, mostly of vertebrates but including also *Drosophila* imaginal discs. The fourth deals with some topics of high contemporary interest: growth and stem cells, regeneration, regenerative medicine, and evolution. To assist readers unfamiliar with the families of genes and molecules that are important in development, they are listed in the Appendix in the context of a short revision guide to basic molecular and cell biology.

Like the previous editions, the new version of *Essential Developmental Biology* differs from its main competitors in four important respects, all of which I 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 in *Xenopus*, 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 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 I 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 I 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 two editions were very well received by both users and reviewers and I hope that the third edition will make this book an even more popular choice for undergraduate and graduate level teaching around the world.

The curse of detail

The principal challenge today is that of exponentially increasing detail. The molecular life sciences seem to double in workforce and output every 10 years or so, and developmental biology has grown at least this fast since its re-founding as a molecular subject in the 1980s. But students' brains do not double in size every 10 years, nor do their courses lengthen. So very serious thought has to be given to what to include and what to leave out.

I have taken the view that the value of model organisms is that they enable experiments to be performed whose results illuminate some general principle of development. However, a lot of recent research has diverged from this strategy and has focused on an ever more detailed molecular analysis of every possible process and organ system in every model organisms. Although the principles of development are common to all animals, it is inevitable that the harder you look, the more differences between organisms you will find. My view is that for a textbook at this level it is not appropriate to cover all the detailed differences between, say, myogenesis in mouse, chicken, *Xenopus,* and zebrafish, so I have focused on just the main themes. In the general field of organogenesis it is also not feasible to cover all of the organs in the vertebrate body. Accordingly, I have selected for inclusion those that are classic developmental biology topics, such as limb development, and those of the greatest medical importance, such as the nervous system, the heart, or the pancreas. Likewise, when considering regeneration, it is not possible to cover every structure in the living world that regenerates to some extent, so I have focused on three classic systems: the planarian, insect limbs, and vertebrate limbs.

New features

Two new chapters in the third edition are devoted to Techniques for Studying Organogenesis and Postnatal Development and Applications of Pluripotent Stem Cells. The Techniques chapter mostly deals with the very important genetic methods in the mouse used for conditional knockouts, induction of transgene activity, and labeling of specific cell lineages. The Applications chapter deals with human embryonic stem cells and induced pluripotent stem cells and their current and future applications. The two chapters on Tissue Organization and Stem Cells and on Growth, Aging, and Cancer, areas of enormous current interest, are both very substantially revised, and place tissue-specific stem cells firmly into the context of normal postembryonic development and cell turnover.

Otherwise, the text has all been rewritten and updated, the grouping of topics has been reorganized to some extent, the further reading has been updated, and errors have been removed. I have also taken the opportunity of the third edition to introduce many photographs. This helps to communicate the remarkable beauty of specimens, which is what motivates many developmental biologists to do their work.

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, I have kept separate the model organism species in Section 2, and for Sections 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. I 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. I 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 gene in more than one species, I 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, I refer respectively to "upregulation" and "activation" for these two situations, and to "repression" and "inhibition" for the situation where expression or activity is reduced.

I am 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, I 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

I am grateful to the Wiley-Blackwell editorial staff, Karen Chambers and Mindy Okura-Marszicky, for their assistance; to Debbie Maizels of Zoobotanica for her excellent artwork; to Kathy Syplywczak for her able production management; to Mike O'Connor and Anne Rougvie of the University of Minnesota who read chapters for me; to Abeer Yakout who helped eliminate some errors; to James Dutton, Lucas Greder, Kevin Lin, and many past lab members for providing photographs; to all my current lab members, who have tolerated the many hours I spend reading and writing; and to my family who have provided unstinting support.

Jonathan M.W. Slack
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About the companion website

There is a companion website available for this book at
www.essentialdevelopmentalbiology.com

On the site you will find:

- Animations
- Figures from the book
- Self-test questions and answers
- Useful website links

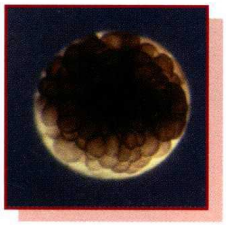
and more

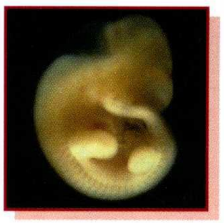
The website is sign-posted throughout the book. Look out for these icons:

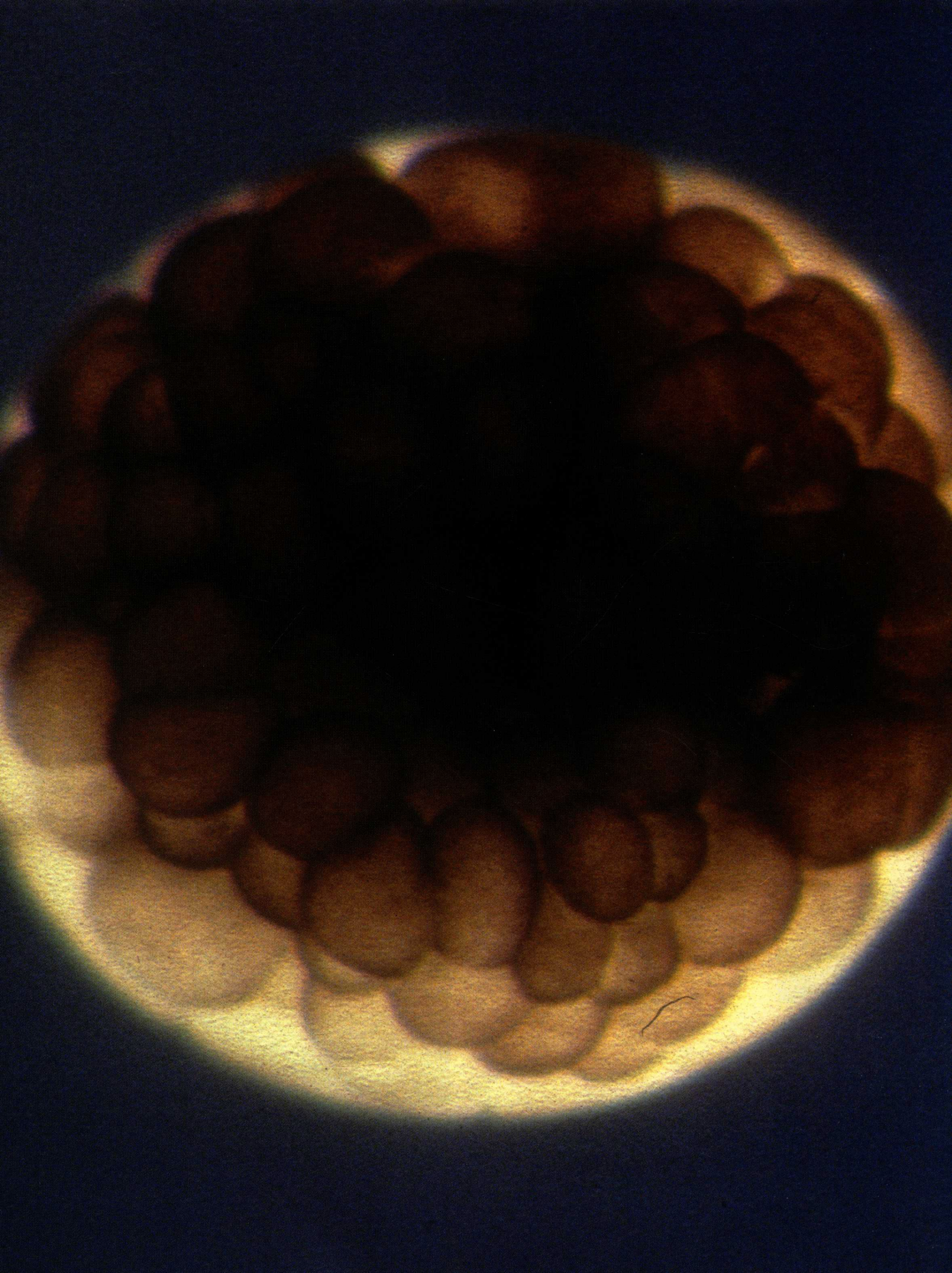
Contents

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 functional cells from stem 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 inducing factors, their receptors, signal transduction pathways, and transcription factors. 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. The first processes of development create a certain simple morphology, 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 synthetic discipline, involving contributions from these three areas of science. When thinking about developmental problems it is necessary to be able to use concepts from these 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 biological 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 30 years later we know a lot and it is possible to write undergraduate textbooks on the subject. Over this period, developmental biology has been one of the most exciting areas of biological research. The dramatic advances came from three main traditions that became fused together into a single world view: experimental embryology, developmental genetics, and molecular biology.

Experimental embryology had been going since the beginning of the twentieth century, when it consisted mainly of microsurgical experiments on embryos of frogs 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 also existed for a long time, but it really flowered in the late 1970s when mass genetic screens were carried out on the fruit fly *Drosophila*, 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.

Molecular biology had 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 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

Essential Developmental Biology, Third Edition. Jonathan M.W. Slack.

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. 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

Some areas of developmental biology have had a significant impact on society in recent decades. ***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 as many as 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 2011, Robert Edwards 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, or 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 potentially 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 probably not 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**. In particular Down's syndrome is due to the presence of an extra chromosome, and there are a number of relatively common abnormalities of the sex chromosomes. These can be detected in cells 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 samples of **chorionic villi**, which may be taken in the early stages of pregnancy. Many more birth defects are due to mutations in genes that control development. It is now possible to screen for some of these, either in the DNA of the parents or that of the embryo or 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 other growth factors, such as the fibroblast growth factors (FGFs) 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 human genetic disease as often a targeted mutation in the mouse can mimic a human disease that arises from non-mutational causes.

Developmental biology has also been the "midwife" of **stem cell** biology. Embryonic stem cells were discovered by developmental biologists and the methods for directed differentiation of these cells depends on the understanding of the normal sequence of embryonic inductions which has been built up by developmental biologists. Stem cell biology has now become a huge science in its own right, with many potential medical applications.

Future impact

Although the past impact of developmental biology is significant, the future impact will certainly be much greater. Some of the benefits are indirect and not immediately apparent. Some, 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 first main area of practical significance is that an understanding of developmental mechanisms will assist the pharmaceutical industry in designing new drugs effective against cancer or against degenerative diseases such as diabetes, arthritis, and neurodegeneration, conditions that continue to cause enormous suffering and premature death. The processes that fail in degenerative diseases are those established in the course of embryonic development, particularly its later stages. Understanding which genes and signaling molecules are involved has provided a large number of potential new **therapeutic targets** for possible intervention. Once the targets have been identified by developmental biology, the new powerful techniques of **combinatorial chemistry** are applied by pharmaceutical chemists to create drugs that can specifically augment or inhibit their action.

Secondly, and as a quite separate contribution to the work of the pharmaceutical industry, various developmental model systems are important as assays. The *in vivo* function of many **signal transduction pathways** can be visualized in *Xenopus* or zebrafish or *Drosophila* or *Caenorhabditis elegans*, and can be used to assay substances that interfere with them using simple dissecting microscope tests. Genetically modified mice have become very important as models for specific human diseases. Because they are looking at the whole organism these assays are more powerful than biochemical assays on cells in tissue culture.

Thirdly, there is the extension of the existing **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 are performed on an individual's genetic makeup, the more likely they are to be denied insurance or particular career opportunities because they have some susceptibility to some disease or other. This is a problem that society as a whole will have to resolve.

Fourthly, there will be a widening application of our understanding of growth and regeneration processes for therapy. For example factors may be developed that could make pancreatic β cells grow, which would be very useful for the treatment of diabetes, or something that could promote neuronal regeneration, which would be useful in treating a variety of neurodegenerative disorders.

Fifthly, there is the application of developmental biology to the production of human cells, tissues, or organs for **transplantation**. This is usually called **cell therapy** if cells or tissues are transplanted, rather than whole organs. At present all types of transplantation are seriously limited by the availability of donors and the ability to make cells, tissues, or even organs on demand has been the principal public justification for funding of stem cell research. The route to replacement envisages their growth from human **pluripotent stem cells**. In a dramatic recent discovery it has been found possible to reprogram normal fibroblasts or other cell types to become pluripotent stem cells (**iPS cells**) through the upregulation of a small number of genes already known to be important for the properties of **embryonic stem cells**. Pluripotent stem cells have the ability to grow without limit in tissue culture, and, when placed in the appropriate environment, to differentiate into all or most of the cell types in the body. Especial interest is shown in methods for making pancreatic **β-cells** for treatment of diabetes, **dopaminergic neurons** for treatment of Parkinson's disease, and **cardiomyocytes** for treatment of heart disease. Not only do pluripotent stem cells hold out the promise of creating these cell types on demand, but it is in principle possible to grow "personalized" cell lines, which would be a perfect immunological match for the patient to be treated.

The new stem cell technology is likely to become 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.

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 pharmaceuticals in the milk of sheep, or vaccines in eggs, and other opportunities will doubtless present themselves in the future.

Further reading

Useful web sites

Society for Developmental Biology: Education section
http://www.sdbonline.org/index.php?option=com_content&task=section&id=6&Itemid=62
The virtual embryo:
http://www.ucalgary.ca/UofC/eduweb/virtualembryo/

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. (2004) *Human Embryology and Developmental Biology*, 4th edn. Philadelphia: Mosby Elsevier.
Schoenwolf, G., Bleyl, S., Brauer, P. & Francis-West, P. (2008) *Larsen's Human Embryology*, 4th edn. New York: Churchill Livingstone.

Textbooks, mainly analytical

Gilbert, S.F. (2010) *Developmental Biology*, 9th edn. Sunderland, MA: Sinauer Associates.
Wolpert, L. & Tickle, C.A. (2010) *Principles of Development*, 4th edn. Oxford: Oxford University Press.

Reproductive technology, teratology, ethics

Braude, P. (2001) Preimplantation genetic diagnosis and embryo research – human developmental biology in clinical practice. *International Journal of Developmental Biology* **45**, 607–611.