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Essential Developmental Biology

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Preface

This book presents the basic ideas and facts of modern developmental biology of animals. Special attention has been given to keeping it compact and concise. It should be suitable as a core text for undergraduate courses from the second to the fourth year, and for beginning graduate courses. The first edition has been “road tested” by myself and by many other instructors, and it has been 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.

Organization

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 section 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 section deals with stem cells and organ development, mostly of vertebrates but including also Drosophila imaginal discs. The fourth section deals with growth, regeneration, and evolution. To assist readers unfamiliar with the 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.

Distinctive approach

This book differs from its main competitors in four important respects, all of which I feel are essential for effective education. Firstly, 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. Secondly, I have avoided all considerations of history and experimental priority because students do not care who did something first if it all happened twenty years ago. Thirdly, on the other hand, I have been careful to stress at all stages why we believe what we do. Understanding does not come from simply memorizing long lists of gene names, so I have insisted that students understand how to investigate developmental phenomena and what sorts of evidence are needed to prove a particular type of result. Finally, 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 interesting but are really separate branches of biology.

Changes to this edition

The first edition was very well received by both users and reviewers and I hope that the second edition will make Essential Developmental Biology an even more popular choice for undergraduate teaching around the world. The changes made for this edition reflect both the requests of users and the changes in the subject matter over the last few years. Users overwhelmingly wanted color in the illustrations, so this has been provided. There is now a glossary at the end which defines all the key terms shown in bold in the text, and each chapter also contains a set of summary bullet points. The web-based materials have been expanded and now include animations in addition to the full set of illustrations used in the book. The users we consulted also tended to want more material on their own favorite topic. This was more difficult to provide as everyone’s favorite topic is different, and to please everyone would have led to an explosion in length that would have ended up pleasing nobody. However some modest additions have been made. There is more on mammalian fertilization, which is always of interest to students. There is more on the heart and the gut, as these are such central topics in human embryology, and there is much recent research progress. There is more on stem cells, growth, and aging, all hot research topics with obvious practical significance. Finally, a new chapter on evolution and development now gives this area
a higher profile than in the first edition. Otherwise, the text has all been rewritten and updated, the grouping of topics has been reorganized to some extent, the references have been rationalized, and errors have been removed.

Developmental biology has become a very detailed and complex subject and this means that inevitably most of the references in an elementary text have to be to reviews. A consequence of this could be that students would never read any original scientific papers, which would be very undesirable. So I have now included boxes with primary references to some key major discoveries. The choice of these references is of course personal and subjective but I hope they will communicate the excitement involved in research to those who look them up. I have probably been even more subjective in my choices of future priorities in the boxes on “new directions for research,” but the object of these is to indicate that the subject is still moving and these boxes may be useful as the starting points for some discussions.

Students sometimes consider developmental biology to be a difficult subject, but this need not be the case as 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 all 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 animal 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 (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 repressive steps, such as the Wnt pathway, cause considerable trouble because of a failure to understand that the lack of something may be just as important as the presence of something. Here, these issues are fully explained in the early chapters, with appropriate reinforcement later on. 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 bare minimum required to explain the workings of a particular process. This sometimes means that various parallel or redundant components are not mentioned, and the latest detail published in Cell is omitted.

Summary of key new features:

- Instructor CD with artwork in downloadable format
- Website including 25 animations, interactive exercises, all text, artwork, and also simple schematic art. Animations are indicated in the margin with the icon. Access is free with purchase of new book (access may also be purchased by visiting www.blackwellpublishing.com and searching for ISBN 1-4051-4646-X)
- New chapters on Tissue Organization and Stem Cells (Chapter 13), Development of Endodermal Organs (Chapter 16), and Evolution and Development (Chapter 20)
- Expanded coverage of mammalian fertilization, the heart, growth control, and aging
- "Classic Experiment" boxes with primary references
- "New Directions for Research" boxes
- End-of-book glossary
- End-of-chapter summaries for quick review
- Numerous new figures, including model organism comparison chart (Chapter 6)
- Four-color used throughout

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 well prepared to enter 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 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.

Acknowledgments

Finally, I should like to thank some people who have been involved with the work: Nancy Whilton who enthusiastically commissioned and guided this edition; Elizabeth Wald who very capably managed the day-to-day details in developing this edition; Debbie Maizels of Zoobotanica for the excellent illustrations; Rosie Hayden, Sarah Edwards, and Brian Johnson who have skillfully handled the complex production; and the numerous reviewers, listed below, who have made many helpful comments on sections of the manuscript. The responsibility for any residual errors is mine and I shall be pleased to hear from readers who discover them.

Reviewers:
Judith E. Heady, University of Michigan-Dearborn
Most importantly, I should like to thank my lab members who have put up with a lot of unavailability on my part, and my family whose patience and support during this long writing process was also invaluable.

Jonathan Slack
Bath, 2005
Section 1

Groundwork
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. As recently as 1980 we knew nothing of these mechanisms, but 25 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. These 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 in existence since the beginning of the twentieth century, consisting mainly of microsurgical experiments on embryos of frogs and sea urchins. These had demonstrated the existence of embryonic induction: chemical signals that controlled the pathways of development of regions 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 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 meant that the mechanisms of development could for the first time be worked out 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 has turned out 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.

Central position in biology

Developmental biology occupies a pivotal position in modern biology. This is because it unites the disciplines of molecular biology, cell biology, genetics, and morphology. Molecular and cell biology tell us about how the individual components work: the inducing factors, their receptors, the signal transduction pathways, the 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, eventually to create a more complex morphology.

So developmental biology is a synthetic discipline in which an understanding of molecular biology, genetics, and morphology...
is necessary. When thinking about developmental problems it is necessary to be able to use concepts from these three areas simultaneously because they are all necessary to achieve a complete picture.

Impact on society

Certain 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 many previously infertile couples to have a baby. Its variants include artificial insemination by donor (AID), egg donation, and storage of fertilized eggs by freezing. It is perhaps less widely appreciated that AID, IVF, embryo freezing, and embryo transfer between mothers is also very important for farm animals. It has 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.

Developmental biology is responsible for an understanding of the chromosomal basis of some 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. 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 in the embryo itself, using molecular biology techniques.

Future impact

Although the past impact of developmental biology is significant, the future impact will be much greater. Some of the benefits are indirect and not immediately apparent. Some, particularly those involving human genetic manipulation or cloning, will 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 human genome is now fully cataloged and sequenced, and so are the genomes of most of the animals used as experimental organisms for studying development. Furthermore techniques are now well advanced for separating and identifying all the proteins in a particular tissue sample (proteomics). This means that it has become much easier to identify genes or gene products associated with particular developmental mutations or diseases, and has led to an increased emphasis on understanding their functions. Developmental biology is a central component of these new disciplines of functional genomics and functional proteomics.

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. As is well known, these conditions 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 will provide 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 can be 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 manipulated mouse embryos are increasingly being 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 by other means.

Thirdly, there is the possibility of using our understanding of growth and regeneration processes for therapy. This has already been done to some extent. For example the hematopoietic growth factors erythropoietin and granulocyte–macrophage colony-stimulating factor (GM-CSF) have both been used in clinical practice for some years to treat patients whose blood cells are depleted by cancer chemotherapy, or for other reasons. In future other factors may also be developed. For example, something that could make pancreatic β-cells grow would be very useful for the treatment of diabetes, or something that could promote neuronal regeneration would be useful in treating a variety of neurodegenerative disorders.

Fourthly, 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 that individual is to be denied insurance or particular career opportunities because of a susceptibility to some disease or other. It also risks the creation of an underclass of genetically “suspect” persons, contrasted with the screened and supposedly “clean” ones. This is a problem that society as a whole will have to resolve.

Fifthly, and even more controversial, there is the possible application of developmental biology to the production of human tissues or organs for transplantation. At present transplantation is seriously limited by the availability of donor organs. There are two conceivable routes to this end. The tissue engineering route involves the growth of the tissue or organ in vitro either from stem cells or from combinations of mature cells that can be cultivated outside the body. 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.

The second route to replacement tissues and organs envisages their growth from human embryonic stem cells (ES cells). This may be possible by improvement of culture conditions or it may turn out also to require genetic modification of the cells. In either case there are potential ethical problems connected with genetic modification of human tissues and with the use of human eggs for a purpose other than conventional reproduction. This issue also intersects with the debate about human cloning. Although there is virtually universal agreement that human beings should not be “copied” by cloning methods (the procedure called reproductive cloning), the majority of scientists do favor the potential use of cloned embryos as a source for tissue grafts. This is called therapeutic cloning and involves growing the ES cells from an egg in which the nucleus has been replaced by one from the individual needing the graft. The potential advantage is that this could be a method for creating a limitless supply of grafts with perfect immunological compatibility. The continuing ethical debate on this matter arises because the procedure technically involves the creation of an embryo for a purpose other than reproduction.

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 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
Zygote: http://zygote.swarthmore.edu/
The virtual embryo: http://www.ucalgary.ca/UofC/eduweb/virtualembryo/
Bill Wasserman’s developmental biology page: http://www.luc.edu/depts/biology/dev.htm

Textbooks, mainly descriptive

Textbooks, mainly analytical

Monograph

Reproductive technology and ethics