国外优秀生命科学教学用书

Essential Developmental Biology

发育生物学基础

影印版

• Jonathan Slack

高等教育出版社
Higher Education Press

图字：01-2002-3318 号

Reprinted from
Essential Developmental Biology
Jonathan Slack

This edition is published by arrangement with **Blackwell Publishing**, Oxford.

图书在版编目（CIP）数据

发育生物学基础＝Essential Developmental Biology/
(英)史莱克(Slack,J.)著. －影印本. －北京：高等
教育出版社，2002.11
ISBN7-04-011467-4

I. 发… II. 史… III. 发育生物学－英文
IV. Q111

中国版本图书馆 CIP 数据核字（2002）第 087054 号

策　　划 王　莉　**封面设计** 王凌波　**责任印制** 韩　刚

出版发行	高等教育出版社	**购书热线**	010-64054588
社　　址	北京市东城区沙滩后街 55 号	**免费咨询**	800-810-0598
邮政编码	100009	**网　　址**	http://www.hep.edu.cn
传　　真	010-64014048		http://www.hep.com.cn
经　　销	新华书店北京发行所		
印　　刷	高等教育出版社印刷厂		
开　　本	787×1092　1/16	**版　　次**	2002 年 11 月第 1 版
印　　张	20.75	**印　　次**	2002 年 11 月第 1 次印刷
字　　数	500 000	**定　　价**	32.30 元

本书如有缺页、倒页、脱页等质量问题，请到所购图书销售部门联系调换。

出 版 前 言

随着克隆羊的问世和人类基因组计划的完成，生命科学成为21世纪名副其实的领头学科，生物高新技术产业逐步成为高科技产业的核心。生物科技和生物产业的发展对世界科技、经济、政治和社会发展等方面产生着深刻的影响，这也是我国赶超世界发达国家生产力水平最有前途和希望的领域。生命科学与技术全方位的发展呼唤高等教育培养更多高水平的复合型科技人才。

为此，教育部在《关于加强高等学校本科教学工作　提高教学质量的若干意见》[教高（2001）4号文件]中提出，高等学校要大力提倡编写、引进和使用先进教材，其中信息科学、生命科学等发展迅速、国际通用性强、可比性强的学科和专业可以直接引进先进的、能反映学科发展前沿的原版教材。教育部高等教育司还于2001年11月向全国主要大学和出版社下发了“关于开展‘国外生命科学类优秀教学用书’推荐工作的通知”，有力推动了生命科学类教材的引进工作。

高等教育出版社对国外生命科学教材进行了充分的调研，并委托教育部高等学校生物科学与工程教学指导委员会的专家教授开展了“引进国外优秀生命科学教材及其教学辅助材料专项研究”，并就国内外同类教材进行了比较，提出了具体的引进教材书目。经过版权谈判，目前我社已经购买了Pearson Education, McGraw-Hill, John Wiley & Sons, Blackwell Science, Thomson Learning, Cambridge University Press, Lippincott Williams & Wilkins等出版的13种教材的影印权，学科领域涉及生物化学、细胞生物学、遗传学、微生物学、生态学、免疫学、神经科学、发育生物学、解剖学与生理学、分子生物学、普通生物学等。这些教材具有以下特点：（1）所选教材基本是近2年出版的，及时反映了学科发展的最新进展，在国际上使用广泛，具有权威性和时代感；（2）内容简明，篇幅适中，结构合理，兼具一定的深度和广度，适用范围广；（3）插图精美、丰富，既有很强的艺术性，又不失严谨的科学性，图文并茂，与正文相辅相成；（4）语言简练、流畅，十分适合非英语国家的学生阅读。其中9种已入选教育部高等教育司推荐“国外优秀生命科学教学用书”。

考虑到中国国情，为了让学生买得起，同时又能让学生看到原版书彩色精美的插图，我们在引进学生用原版教材时，一方面采用黑白影印，最大限度地降低定价，另一方面随书附赠含有原书彩色插图的光盘，以充分体现原教材的风格、特色，为读者提供方便。

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高等教育出版社

2002年11月

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Essential Biology	生物学导论

Preface

This book presents the basic ideas and facts of modern developmental biology at undergraduate level. It is intended to support teaching by lectures, practicals and tutorials, the content corresponding approximately to the ground covered over 3 years of developmental biology courses at the University of Bath. As it is an undergraduate textbook, special attention has been given to keeping it compact and concise. A basic knowledge of cell biology and molecular biology is assumed, but no prior knowledge of animal structure or histology should be necessary.

I have adopted a conceptual approach to the subject with no attempt to provide a historical perspective. The history of embryology is very interesting but the subject has now reached a degree of maturity such that it can be fully understood without knowing how the ideas were originally arrived at, or who did which particular experiment first. At the same time, I have tried to avoid presenting developmental processes as bare facts to be learned. Throughout, I give attention to the appropriate methods for investigation of a problem, and the types of evidence required to arrive at a particular conclusion. This should enable students to apply the principles of what they have learned rather than simply recite the factual content.

The book is arranged in three sections. The first introduces the basic concepts of the subject. 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, tissue organization and regeneration. This section is mainly focused on vertebrates although a chapter is also included on the *Drosophila* imaginal discs. The order of topics is intended to represent a logical progression. However, in real life teaching it is unlikely that anyone would learn general principles before being exposed to some specific examples. So section 1, and particularly Chapter 3, which lists important molecules, is not intended to be read sequentially by the beginning student, but rather to be dipped into in conjunction with the study of the specific topics in later sections.

Developmental biology is now very complex and detailed and it is essential to select those topics for consideration that are of central importance and are reasonably well understood. This has meant that many organ systems and several organisms used for research are not included. In particular I have not dealt with plant development as I feel that the mechanisms involved are rather different from those used by animals. Despite the many omissions, the principles presented here should enable students to understand the subject and to be able to read independently on other aspects of animal development if they wish. Because this is an introductory book, many of the references given are to reviews rather than to original papers but these will lead on to the primary literature where required. Several websites are also listed as sources, as these are particularly useful for tabulated data on such things as gene expression patterns and mutant phenotypes. Throughout, key terms are presented in **bold** type on first mention. According to the usual convention, the names of genes or of their messenger RNAs are written in *italic* but names of their protein products are in Roman type.

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 experi-

ments, and a consistent nomenclature is adopted (e.g. 'anterior' is used throughout rather than 'rostral' or 'cranial'). Most other books 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 here kept separate the animal species in section 2, and for section 3 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 forms (e.g. loss of function, constitutive or dominant negative alleles), 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. Finally, I have tried to keep the overall level of detail, in terms of the number of genes, signalling systems and other molecular components, to the bare minimum required to explain the workings of a particular process, even though this may mean that various parallel or redundant components are not mentioned.

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 programme 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, 4-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.

Finally I should like to thank some people who have been involved with the work: Anne Stanford of Blackwell Science who originally invited me to write it; Debbie Maizels of Zoobotanica for the excellent illustrations; and Andrew Ward, Robert Kelsh, Elizabeth Jones, Jonathan Cooke, Phil Ingham, David Ish Horowicz, Julie Ahringer, Jonathan Bard and Jeremy Brockes for 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.

Jonathan Slack
Bath
2000

Contents

Section 1

Groundwork

1

The Excitement of Developmental Biology

Where the subject came from

As recently as 1980 we had very little idea how animals developed. Twenty years later we have a very good idea 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 progress in understanding came from three main sources: experimental embryology, developmental genetics and molecular biology.

Classical **experimental embryology** had been going 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. 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.

Molecular biology had started with the discovery of the 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 many 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 gene regulation.

Central position in biology

Developmental biology occupies a pivotal position in modern biology. This is because it unites the disciplines of **molecular biology**, **genetics** and **morphology**. Molecular biology tells 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 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 signalling 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 animal structure 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.

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.

Several organisms, including humans, will soon have their genomes completely catalogued and sequenced. At this point, attention will shift from the identification of genes to understanding their functions, and developmental biology is a central component of this new science of **functional genomics**.

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. These conditions cause enormous suffering and premature death. The life processes that fail are those established in the course of embryonic development, particularly its later stages. Understanding which genes and signalling 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 can be applied as quick and simple **assays** for particular groups of genes or molecular pathways and can be used to assay substances that interfere with them. In particular the *in vivo* function of many **signal transduction pathways** can be assayed in *Xenopus* or *Drosophila* or *Caenorhabditis elegans*, using simple dissecting microscope tests.

Thirdly, there is the possibility of using our understanding of **growth and regeneration processes** for **therapy**. This has already been done to a small extent. For example the haematopoietic

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 a 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. 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, there is the possible application of developmental biology to the production of human organs for **transplantation**. There are two conceivable routes to this end. The first involves the growth of the organ *in vitro* from stem cells, and the second envisages the genetic reprogramming of an egg to achieve the same objective. Both involve potential ethical problems connected with genetic modification of human tissues and with the use of human eggs for a purpose other than conventional reproduction.

Finally, we should not overlook the likely applications of developmental biology to **agriculture**. The ability to genetically redesign our farm animals and crop plants must eventually produce a cornucopia of possibilities. The scope is greatest for plants where a long-term dream might be to make an 'all-purpose' crop plant. This might have the ability to fix nitrogen, so that it required no fertilizer; it might produce an edible tuber underground, a useful fibre from the stems, an edible fruit or seed, and perhaps also some desirable natural products from the leaves. With 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 are being 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

Balinsky, B.I. & Fabian, B.C. (1981) *An Introduction to Embryology*, 5th edn. Philadelphia: Saunders.
Gilbert, S.F. & Raunio, A.M. (1997) *Embryology: constructing the organism*. Sunderland, MA: Sinauer Associates.
Hildebrand, M. (1995) *Analysis of Vertebrate Structure*, 4th edn. New York: John Wiley & Sons.
Larsen, W.J. (1993) *Human Embryology*. New York: Churchill Livingstone.

Textbooks, mainly analytical

Browder, L.W. (1998) *Developmental Biology*, 4th edn. Orlando, Florida: HB Coll Publishers.
Gilbert, S.F. (2000) *Developmental Biology*, 6th edn. Sunderland, MA: Sinauer Associates.
Kalthoff, K. (1996) *Analysis of Biological Development*. New York: McGraw-Hill.
Slack, J.M.W. (1991) *From Egg to Embryo*, 2nd edn. Cambridge: Cambridge University Press.
Wolpert, L. (1998) *Principles of Development*. Oxford: Oxford University Press.

2

General Problems of Development

Developmental biology is the science that seeks to explain how the **structure** of organisms changes with time. Structure, which may also be called **morphology** or **anatomy**, encompasses the arrangement of parts, the number of parts and the different types of parts. Parts may be large, such as whole organs, or small, down to the level of the organization of individual cells.

Development happens most obviously in the course of **embryonic development** as the fertilized egg develops into a complete organism. This book deals mainly with embryonic development. But it should not be forgotten that development can also occur in postembryonic life. Many animals have life cycles involving a larval stage that is specialized for feeding and/or for dispersal. The larva will at some stage undergo a **metamorphosis** in which the body is remoulded to a greater or lesser extent to form the adult. Of the model organisms considered in this book, *Drosophila* shows a drastic metamorphosis during which most of the adult body is formed from **imaginal discs** laid down in the larva. *Xenopus* also undergoes metamorphosis from a tadpole to the adult frog.

Some animals are capable of **asexual reproduction** by forming buds, and this is usually associated with the ability to **regenerate** large parts of the body after loss caused by predators. This is true for example of many **hydroids** and **planarian worms**. Regenerative ability is less evident in higher animals but some amphibians have the ability to regrow limbs and tails after amputation, and even in mammals there is a certain ability to repair tissue damage following **wounding**.

Each of these examples of development involves similar problems and they can loosely be classified into four groups:

1 Regional specification deals with how pattern appears in a previously similar population of cells. For example, most early embryos pass through a stage called the **blastula** or **blastoderm** at which they consist of a featureless ball or sheet of cells. Somehow the cells in different regions need to become programmed to form different body parts such as the head, trunk and tail. This often involves processes of intercellular signalling, otherwise called **embryonic induction**, leading to the activation of different combinations of regulatory genes in each region. Similar processes of regional specification occur later in development during the formation of individual organs, or still later during the course of metamorphosis or regeneration.

2 Cell differentiation refers to the mechanism whereby different sorts of cells arise. There are more than 200 different specialized cell types in a vertebrate body, ranging from epidermis to thyroid epithelium, lymphocyte or neuron. Each cell type owes its special character to particular proteins coded by particular genes. The study of cell differentiation deals with the way in which these genes are activated and how their activity is subsequently maintained.

3 Morphogenesis refers to the cell and tissue movements that give the developing organ or organism its shape in three dimensions. This depends on the dynamics of the cytoskeleton and on the mechanics and viscoelastic properties of cells. It is less well understood than **1** and **2**.

4 Growth refers to increase of size. Although more familiar to the lay person than other aspects of development, it is currently the least well understood aspect in terms of molecular mechanisms.

With a few exceptions, such as the lymphocytes of the immune system, all the different cell types in the animal body retain a complete set of genes. This means that the **regulation of gene activity** is important for all four processes and occupies a central position in developmental biology. Many

techniques for the study of gene expression are described in Chapter 3. The best evidence that all cell types retain a complete set of genes is derived from experiments on the cloning of animals from the nucleus of a single cell.

Nuclear totipotency, cloning of animals

In any animal the sperm and eggs and their precursor cells are known as the **germ line**. All other cell types are called **somatic cells**. The germ-line cells have to retain a full complement of genes otherwise reproduction would be impossible. It is generally accepted that virtually all the somatic cells in animals also contain the full complement of genes and that the differences between cells are due to the fact that different subsets of genes are active. The total DNA content of most somatic cell types is the same; and when examined for the presence of a particular gene by Southern blotting, or by the polymerase chain reaction, DNA from all tissues gives the same results.

The most persuasive evidence has been obtained from experiments in which a whole animal is created using a single nucleus taken from a somatic cell. Because most genes will be required at some stage of development, the fact that a differentiated cell nucleus can support the whole process implies that all the DNA is still present in that nucleus. The experimental procedure is known as **cloning**, but it should be remembered that the term cloning is also applied to the molecular cloning of genes, and to the cloning of cells in tissue culture, particularly important in the production of monoclonal antibodies.

Many types of animal embryo, including frogs, rabbits, cows, sheep and mice, can be cloned by transferring a nucleus from an early embryo cell (a blastomere) back into a fertilized egg whose own nucleus has been removed (Fig. 2.1). In such experiments it is important to have some means of distinguishing an animal arising from the donor nucleus from one arising from the original egg nucleus, in case it was not properly removed or destroyed. This is known as a **genetic marker**. For example the donor nuclei in the frog experiments were often taken from albino embryos lacking the gene for pigment synthesis. Only if the embryos

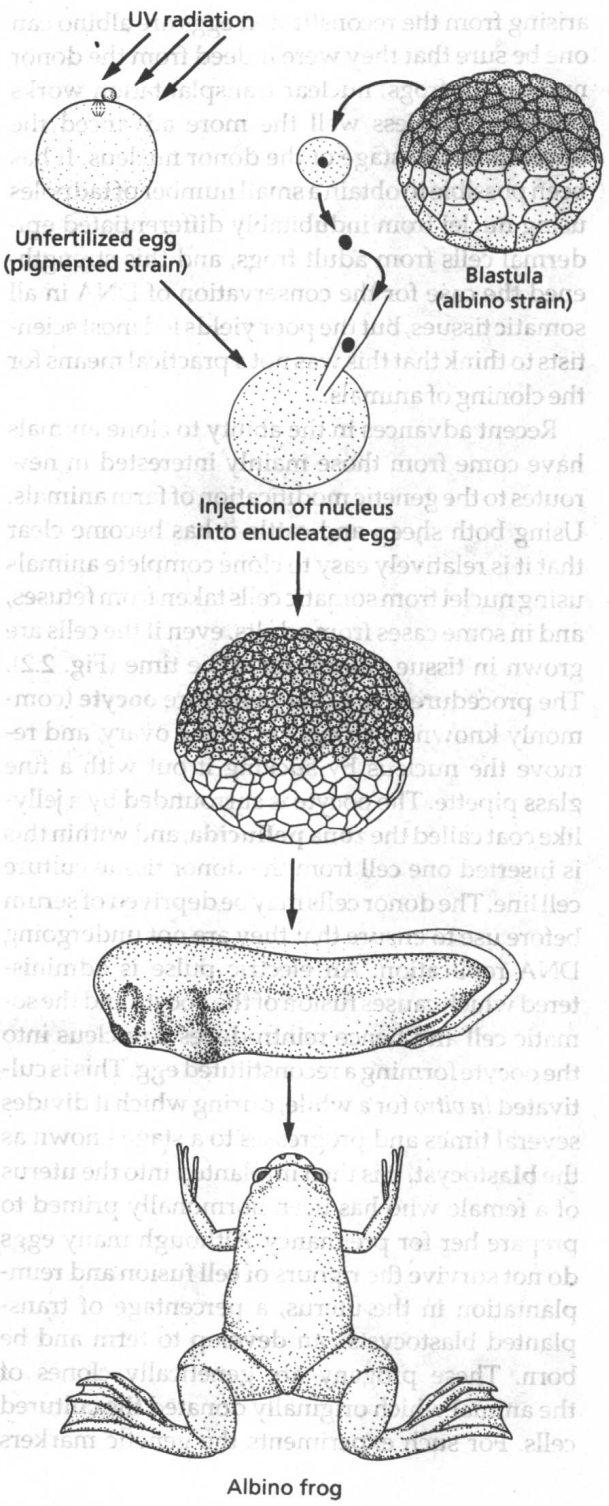

Fig. 2.1 Cloning of frogs from blastula nuclei.

arising from the reconstituted eggs are albino can one be sure that they were indeed from the donor nucleus. In frogs, nuclear transplantation works progressively less well the more advanced the developmental stage of the donor nucleus. It has been possible to obtain a small number of tadpoles using nuclei from indubitably differentiated epidermal cells from adult frogs, and this strengthened the case for the conservation of DNA in all somatic tissues, but the poor yields led most scientists to think that this was not a practical means for the cloning of animals.

Recent advances in the ability to clone animals have come from those mainly interested in new routes to the genetic modification of farm animals. Using both sheep and cattle it has become clear that it is relatively easy to clone complete animals using nuclei from somatic cells taken from fetuses, and in some cases from adults, even if the cells are grown in tissue culture for some time (Fig. 2.2). The procedure is to isolate a mature **oocyte** (commonly known as an **egg**) from the ovary, and remove the nucleus by sucking it out with a fine glass pipette. The oocyte is surrounded by a jelly-like coat called the **zona pellucida**, and within this is inserted one cell from the donor tissue culture cell line. The donor cells may be deprived of serum before use to ensure that they are not undergoing DNA replication. An electric pulse is administered which causes fusion of the oocyte and the somatic cell and hence reintroduces a nucleus into the oocyte forming a reconstituted egg. This is cultivated *in vitro* for a while, during which it divides several times and progresses to a stage known as the **blastocyst**. It is then implanted into the uterus of a female who has been hormonally primed to prepare her for pregnancy. Although many eggs do not survive the rigours of cell fusion and reimplantation in the uterus, a percentage of transplanted blastocysts can develop to term and be born. These progeny are genetically clones of the animal which originally donated the cultured cells. For such experiments the genetic markers

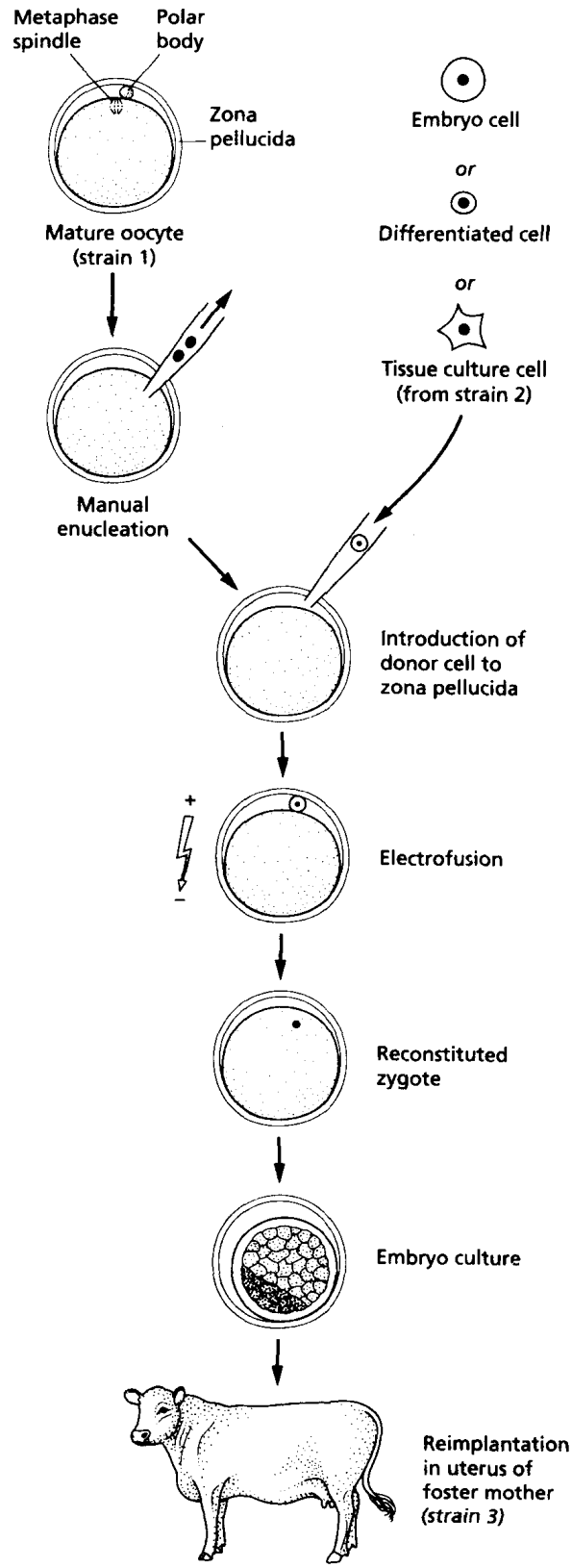

Fig. 2.2 Cloning of mammals. The genetic material may come from a somatic cell or a tissue culture cell.

need to distinguish the cell line from both the recipient egg donor, in case the egg nucleus was not properly removed, and the foster mother, in case of accidental pregnancy, and for this purpose DNA polymorphisms distinguishing the relevant animal breeds are detected using the polymerase chain reaction.

There are in fact some well-known exceptions to the principle that all somatic cells contain the same genes. The antibody-forming genes of B lymphocytes and the T-cell receptor genes of T lymphocytes are known to undergo rearrangement at the DNA level and lose some genetic information in the process. Certain nematodes, although not *Caenorhabditis elegans*, shed chromosomes from some cell lineages during development. There are also a few examples where total copy number of genes may be altered. **Polyploidy**, where the whole chromosome set is doubled or quadrupled, can occur in some mammalian tissues such as the liver. **Polyteny**, where DNA replication occurs repeatedly without chromosomal division, leading to giant chromosomes, occurs in some tissues in *Drosophila*. However, in general the activity of genes is regulated at the level of transcription and subsequent events, and not at the level of the DNA itself.

Further reading

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