Cellular Interactions in Animal Development

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Preface

During the past ten to fifteen years, Cell Biology has come to be recognized in many Universities as a new branch of biology. It could be argued, however, that nearly all of biology is concerned with the study of cells, either individually or in complex groupings which we call organisms. One can re-frame many courses in biology for undergraduates, and one could rewrite many textbooks, under titles which emphasize the 'cellular' aspects of the subject. But besides this, we now have far more information to add which is based on experimental studies of cells in tissue culture, where observations can often be made on individual cells for a long period of time. As a result of advances in techniques for observing living cells, we now know far more than we did fifteen years ago about how cells move, how they make contact with a surface and with each other, how they interact in small groups and what metabolic processes they undergo. In addition, studies with the electron microscope have enabled us to see much more detail of the changes in structure that take place as cells move, interact and differentiate. Nevertheless, having seen these extra details in individual cells, we still have to look back at the larger groups and whole organisms in which they normally function, in order to see the full significance of all these cellular events. One cannot get far in ones studies, if one remains a '*single*-cell biologist'.

I have never regarded myself as a cell biologist. Hence it was with some reluctance that I eventually agreed to write a book under the present title. For a long time I hoped to find a co-author (or, better still, a substitute author!) who would be able to provide the necessary emphasis on cells as biological entities. Failing this, I still hoped to find an expert on invertebrates, to contribute more examples of cellular interaction in this vast majority of the animal kingdom. However, neither hope was realized and I alone, a mere vertebrate embryologist, have had to try to discuss the whole range of cellular interactions in animal development. As a result, I have followed my natural 'homing' instincts and have orientated most of the discussions towards groups of embryonic cells, whole tissues and whole embryos. This has left many gaps, I know, and several readers may feel that the mechanisms of cell-interaction, as studied in tissue culture, are not adequately discussed. However, first- and second-year science and medical students whose courses include either 'cell biology' or 'developmental

biology' may find in these pages an apéritif to their future interests, and the extensive bibliography should help to satisfy their further appetites.

This book is going to press at a time of industrial setbacks which may cause a long delay in its publication: hence there will inevitably be some out-of-date statements in it, if research in cell and developmental biology continues at its present rate. However, the students of next year or the year after will always, I hope, be alive to the possibility of new findings in these subjects, and if any of my over-pedestrian remarks stimulate them to look up the latest research papers, I shall have achieved one of my main objects, which is to provoke thought, criticism and a thirst for more knowledge in this fascinating field of developmental biology.

I owe a great deal of thanks to colleagues in Britain and abroad who have taken an interest in this book. Several authors have kindly provided me with original illustrations from their own work: these are acknowledged below. Many friends have helped me by discussing certain points and by reading parts of the manuscript. I owe particular thanks to Dr Ruth Bellairs and Dr Jim Dodson, who each nobly read through the whole book in its first draft, and offered a number of helpful criticisms. I should also like to thank Dr Brian Pickering and Dr Roger Taylor for their comments on Chapters 10 and 11.

I am indebted to the following authors and publishers for several of the illustrations used in this book.

Professor W. Beermann and Springer-Verlag, Berlin-Heidelberg-New York (Plate 1.1); Professor R. A. Flickinger (original of Plate 1.2); Dr S. Pegrem (original of Plate 1.3a); Dr V. Gabie and the Editor of *Acta Sbryologiae Experimentalis* (Plate 1.3b); Professor A. S. Curtis (original of Plate 1.4); Dr B. Mintz and the Company of Biologists Ltd. (Plate 2.1); Dr R. Bellairs (original of Plate 2.2); Dr R. Presley (original of Plate 3.2); Professor M. Mitchison and the Company of Biologists Ltd. (Plate 3.3); Dr M. Tegner and the American Association for the Advancement of Science (Plate 3.4); Dr D. Szolossi and the Rockefeller University Press (Plate 4.1); Dr J. van den Biggelaar (original of Plate 4.2); Dr M. Kalt and the Company of Biologists Ltd. (Plate 4.3); Mrs P. Hyatt (original of Plate 5.1); Dr P. Flood (original of Plate 6.1); Dr T. E. Schroeder and the American Society of Zoologists (Plate 6.2); Dr R. DeLong and Academic Press Inc., New York (Plate 9.1); Professors G. Veneroni and M. Murray and the Company of Biologists Ltd. (Plate 9.2); Dr S. Bradley and Cambridge University Press (Plate 9.3b); Dr R. Hauser (original of Plate 9.4) and Professor L. B. Arey and W. B. Saunders Co., Philadelphia (Plate 11.1).

Finally, I should like to thank Messrs Chapman and Hall Ltd. for providing some professional help with the line drawings.

Elizabeth M. Deuchar

Bristol, January 1974

Contents

Introduction

The scope of this book

This book attempts to provide students of animal biology with some insight into the variety of mechanisms by which the cells within an animal's body interact with one another and exert controls over each other's development and differentiation. Far more is known about cellular interactions and development in vertebrates than in invertebrates. This is mainly because vertebrate embryos are on the whole larger and easier to study under laboratory conditions than are those of invertebrates. Hence, all through this book, most has been said about vertebrates. Reference has been made to invertebrates when there are specially interesting features to note.

This book is written from the point of view of the embryologist, or 'developmental biologist' as he may now prefer to be called. Students who have not done any animal embryology will find it necessary to read some introductory book on this subject, in order to understand all the terms used in the following pages. For briefer, lighter reading, a book such as *Principles of Development and Differentiation* by C. H. Waddington, or *Living Embryos* by Jack Cohen, might be suitable. Those who have time for more comprehensive reading will find B. I. Balinsky's *Introduction to Embryology* very useful. Fuller details of these books are given in the Bibliography on pp. 256–283.

1 Cells in the developing organism: some general considerations

Cells are the units which make up the body of an animal or plant. Under special conditions, individual cells may be capable of life on their own, but normally in the intact organism they are mutually dependent for their survival and maintenance. In this book we shall deal with animal and not plant cells, and shall be considering only the Metazoa (multicelluar animals), not the Protozoa (in which each organism consists of only one cell), for it is only in the Metazoa that one can discuss cellular interactions within a single developing organism.

The cells of metazoan animals have many different shapes, sizes and functions: the more complex the structure of the animal, the greater is the variety of cells which compose it. The cells are grouped into larger functional units, the tissues and organs, and within each tissue or organ, particularly close cellular interactions take place. Tissues and organs also interact with each other however, thus ensuring that all the functions essential to the life of the organism are carried out efficiently. This continual co-ordination of functions in all its cells is essential to the life and integrity of every animal, from the moment that it starts to become multicellular when the fertilized egg divides into two cells, right through development, adulthood and ageing. At all stages in its life history an animal's cells are interacting, normally in such a way as to promote resistance to any adverse environmental conditions that may arise, and to make good any damage or loss of cells that may occur through external agencies. In later life, some of the cellular co-ordination processes

may begin to fail, so that death from injuries or disease is more likely to occur. But in the early embryo there are remarkable powers of recovery by means of cellular interactions, even after the most drastic experimental procedures.

In this book we shall concentrate on those cellular interactions that occur during embryonic and juvenile stages of life. Before we define the kinds of interactions that may occur, however, we must first give critical consideration to some of the basic 'facts' that are usually accepted as true of the cells in an animal at early embryonic stages.

1.1 The problem of identity of genotype in different cells

It is a commonly accepted generalization that all the cells in any one embryo contain identical sets of *genes,* the factors inherited from the parent organisms via the chromosomes in the nuclei of the egg and the spermatozoön. It is assumed that from the zygote (fertilized egg) stage onwards, at each cell division the daughter cells receive identical sets of genes, since they normally receive identical sets of chromosomes. What evidence have we for this assumption, however? None of it is very direct or conclusive. To start with, there are a few known exceptions to the rule that identical sets of chromosomes pass into all cells during early embryonic cell divisions. In the nematode worm *Ascaris,* and in Chironomid flies, certain chromosomes are lost from all except the future germ cells. In other species of the insect order Diptera, cells of certain tissues such as the salivary glands acquire giant, 'polytene' chromosomes in which all the gene loci have been replicated several times. In all these cases, cells from different tissues clearly do *not* contain identical sets of chromosomes and genes. Even in those animals where there are no gross chromosomal differences between the different cells, we are not in a position to be sure from direct observations that all the genes on corresponding chromosomes in all cells remain identical throughout embryonic development. Even the highest power of an electron microscope does not show enough detail of chromosome structure for every 'cistron' (the active unit of the gene that codes for a polypeptide) to be identified and compared with those of other cells. We have to rely, then, on indirect evidence to support the assumption that there are normally identical sets of genes in all the cells of any individual embryo.

An important line of indirect evidence comes from nuclear trans-

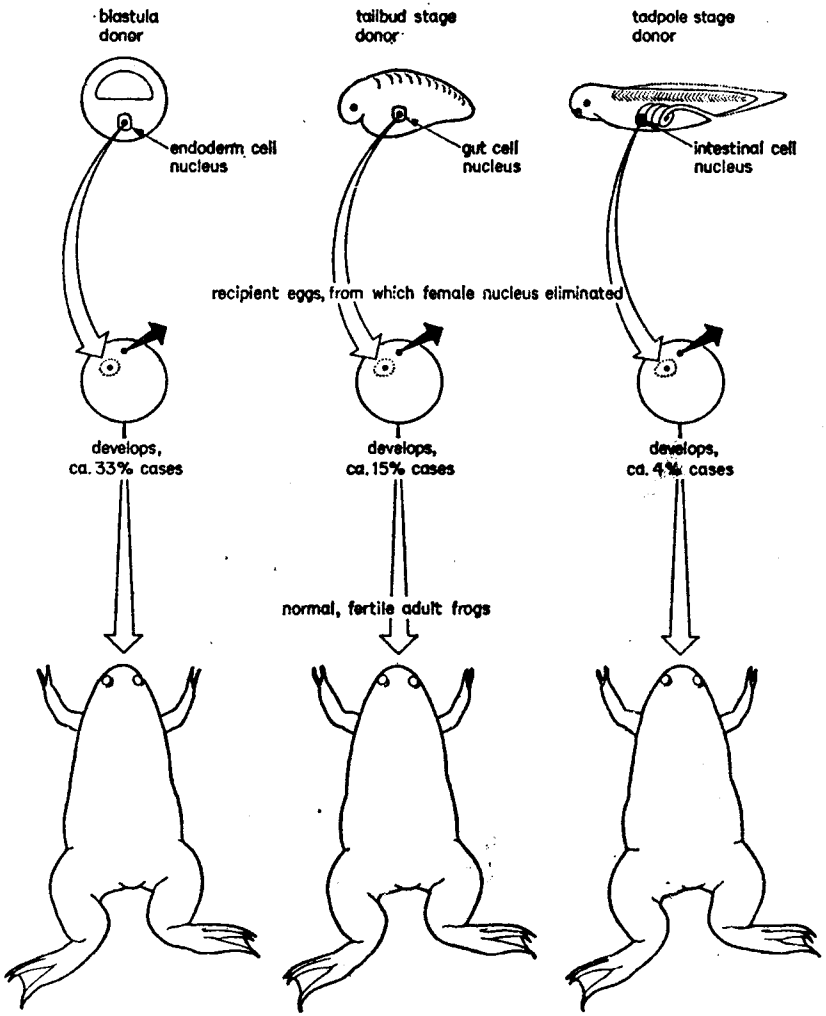

Fig. 1.1 Nuclear transplantation into amphibian eggs.

plantation experiments. Illmensee's work (1972) on *Drosophila,* added to the work of Briggs and King in the 1950s on the American frog *Rana pipiens,* and that of Gurdon and his collaborators in the 1960s on the South African frog *Xenopus laevis,* have shown that nuclei from the cells of quite advanced embryos or even from tadpoles can, when transplanted back into an egg whose own nucleus has been removed, set off normal development and give rise to normal adult individuals in several cases (Fig. 1.1). This shows that all the genes

essential for normal development of the embryo and larva (tadpole) were still present in the transplanted nucleus: none had been lost as the various tissues became differentiated and specialized for different functions in the donor animal. The transplanted nucleus had retained the same genetic potentialities as the zygote nucleus.

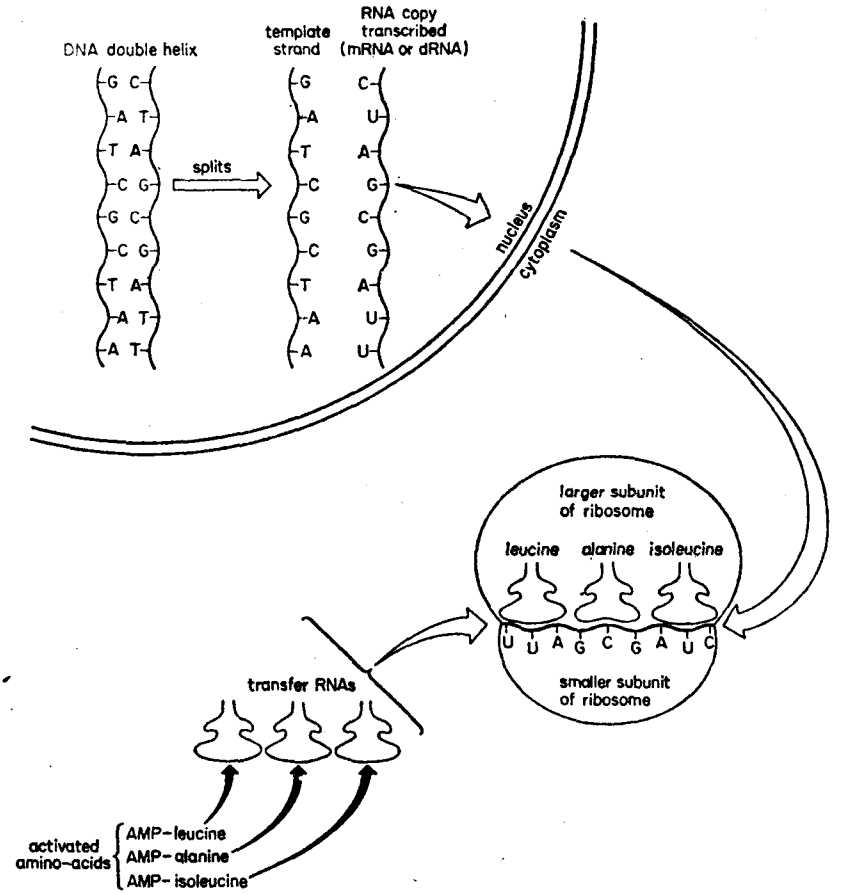

Fig. 1.2 Simplified representation of the genetic control of protein synthesis. A single-stranded DNA template is transcribed as dRNA, which enters the cytoplasm and becomes attached to the smaller subunit of a ribosome. Cytoplasmic amino acids, after activation by attachment to AMP, become attached to small RNA molecules called 'transfer RNA', which carry them to the larger subunit of the ribosome and attach them to suitable triplets of bases (codons) on the dRNA. Adjacent amino acids become bonded together and a polypeptide is thus formed. Several ribosomes may combine as 'polysomes' and thus facilitate the synthesis of larger protein molecules.

If we accept, however, that all cell nuclei of an embryo or larva have identical genetic potentialities, it is not easy to account for the fact that cells differ in form and function, except by assuming that different genes are in some way inactivated in different cells. If, for instance, the genes for haemoglobin production and for myosin synthesis are present in all cells, it is hard to explain why all cells do not become red blood cells or muscle cells, unless one assumes that the haemoglobin and myosin genes are inactivated in all except a very limited number of embryonic cells. But again, the actual evidence for this assumption is not yet very substantial. It all hangs, too, on acceptance of the current hypothesis for gene action. According to this, DNA of active genes is transcribed as 'messenger' RNA (now usually called dRNA), and the sequence of bases in this dRNA determines the order in which amino acids are assembled into a polypeptide on the ribosomes of the cytoplasm. (see Fig. 1.2). This hypothesis has stood up to several experimental tests, but cannot yet be said to be proved beyond all doubt.

On the hypothesis just stated, one could argue that the mere presence of different proteins in different cells must imply that different genes are being transcribed – i.e. that other different genes are inactivated too. A more practical line of approach, however, is to look for differences in the base sequences of dRNA extracted from different types of embryonic cell. If there are differences, this will imply that different genes have been transcribed in the various cells. This experiment was in fact tried by Paul and Gilmour (1968), using molecular hybridization as a test of the degree of similarity of dRNAs from different tissues of the rabbit embryo. The principles of this method are explained in Fig 1.3. Many people now doubt the reliability of some hybridization techniques, however, so although Paul and Gilmour did find differences in the hybridization properties of dRNAs from different tissues, their results are not considered to be conclusive.

Another type of evidence that different genes may be active in different tissues of an individual organism is the presence of 'puffs' at various points on the polytene chromosomes of insects. Plate 1.1 shows such a puff described by Beermann (1973). The puffs lie at different points on the chromosomes in cells of different tissues, and at times such as moulting and metamorphosis, new puffs appear. It has been shown by labelling the cells with radioactive uridine, which is taken up into newly-synthesized RNA, that there is par-

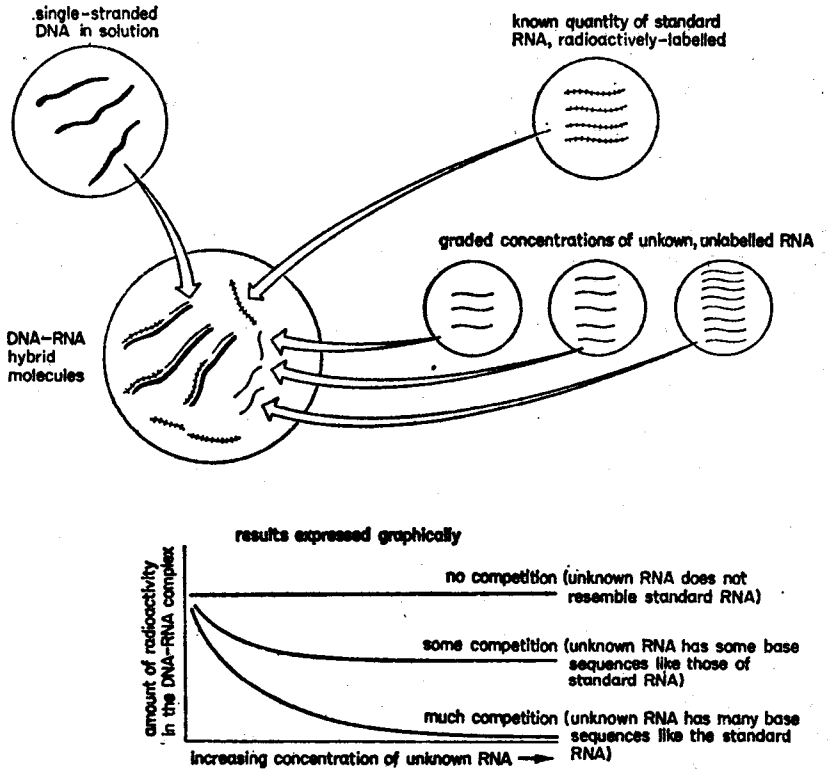

Fig. 1.3 Principles of competitive hybridization experiments. A known quantity of standard, radioactively-labelled RNA is added to single-stranded DNA in solution. Sequences of bases which are complementary to those on the DNA will hybridize with it, forming radioactive DNA-RNA hybrid molecules which may be collected and their radioactivity measured. The experiment is then repeated with the addition of increasing concentrations of the unknown RNA which is to be compared with the standard, and which is not radioactive. The more base sequences it has in common with the standard RNA, the more it will compete with it for hybridization, resulting in less radioactivity in the RNA-DNA hybrid molecules (see graphs.)

ticularly active RNA synthesis in the puff regions. This suggests that they are sites where genes are being transcribed most actively. However, the fact that puffs have been seen in these giant chromosomes of insects cannot be taken as evidence for what happens in all other animals. Labelling work has been carried out also on the chromosomes of amphibian embryos, using radioactive thymidine which is taken up during DNA synthesis, and this has shown that some regions of certain chromosomes replicate later than the rest. Stambrook and Flickinger (1970) compared the locations of late-

replicating regions in some easily-recognizable chromosomes of the frog *Rana pipiens*, and they found that in different tissues of the embryo, these regions differed in number and in distribution. Some of their findings are shown in Plate 1.2. It is rather a remote argument, however, to suggest that the late-replication has much bearing on which genes are transcribed as RNA in the different tissues.

The argument that some genes are inactivated (though still present) in certain embryonic cells is one way of explaining why cells produce the different proteins which are the basis of their differences in structure and function. Some have argued that there could be a process of selection of dRNA types in each cell, so that although all genes were transcribed, only some dRNAs were able to function. This argument requires that there is some different cytoplasmic material in different cells, to do the selecting, however: and to explain the origin of the cytoplasmic differences one has again to revert to possible differences in gene activity: so it becomes a circular argument.

If we are now prepared to accept, in the absence of any better hypothesis, that different genes are inactivated in different embryonic cells, the next question that has to be answered, is what agents inactivate the genes? Jacob and Monod (1961) envisaged the existence of 'regulator' genes, producing substances that inhibited the action of neighbouring genes. Others have considered the possibility that chromosomal proteins 'wrap round' the DNA molecule at certain points. It has been suggested that histones, which form a high proportion of chromosomal protein, do this. But no one has yet been able to explain convincingly how *different* parts of the DNA could be so obscured, in different cells. An extension of Jacob and Monod's idea is to suggest that agents from outside the nucleus, or even outside the cell, may cause inactivation of some of its genes. Gurdon and his collaborators argued from their nuclear transplantation work that the egg cytoplasm was responsible for *reactivating* the genes in the transplanted nucleus. Substances which pass into the cytoplasm of a cell from outside, perhaps coming from an adjacent cell, may therefore be thought of as possible agents for the activation or inactivation of certain genes in the cell. This is thought to be the basis of many influences on cell differentiation that are exerted by neighbouring cells.

We are now in a position to try and define some of the ways in