FROM EGG TO EMBRYO

DETERMINATIVE EVENTS IN EARLY DEVELOPMENT

J. M. W. SLACK

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Preface

This book is an enquiry into the mechanisms by which the spatial organisation of an animal emerges from a fertilised egg. It is intended for all students, teachers and research workers who are interested in embryos.

It is divided into three parts. The first two chapters introduce the problem of regional specification and attempt to define the meanings of embryological terms which are used in the remainder of the book. This is necessary because terms such as 'induction', 'regulation' or 'polarity' are often used but rarely defined and many controversies have arisen as a result of unnecessary misunderstandings.

The next four chapters give an overview of the experimental evidence which bears on the processes of cellular commitment from the time of fertilisation to the formation of the general body plan. The animal types considered are those on which most experimental work has been done: amphibians, insects, other selected invertebrates, the mouse and the chick. This is a general survey rather than a detailed review but sufficient references are provided to enable interested readers to pursue the topics in greater depth.

The last four chapters attempt to generalise the problems and to investigate the extent to which they have been solved by the theorists and the model-builders. In particular several 'gradient' models are examined and assessed in terms of the experimental evidence. This section has been written principally for non-mathematical readers although a few differential equations are provided for those who are interested. Many of the models are relevant to late development and to regeneration as well as to early development, but the focus of the book has been kept on early development because this provides for maximum conceptual unity without undue length.

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Part I

Introduction and definitions

1

Regional specification in animal development

This book is about how an egg becomes an animal. Attention will be concentrated on *early* development because this is the time at which the important events are happening. As everyone knows the human gestation period is about 9 months long, but it is not so commonly appreciated that the basic body plan of the embryo becomes established during the very short period from 1 to 4 weeks after fertilisation. During this time an apparently homogeneous group of cells, the inner cell mass of the blastocyst, becomes transformed into a miniature animal consisting of central nervous system, notochord, lateral mesoderm, somites, branchial arches, integument and gut. All of these parts contain specific types of cell and all lie in the correct positions relative to one another. In later development there is a good deal of growth and of histological differentiation of the organs, and the specifically human, rather than the general vertebrate, characteristics of the organism become established. However, all this takes place on the framework of the basic body plan which was laid down in early development. As Wolpert has emphasised, it is not birth, marriage or death, but gastrulation which is truly the most important time in your life.

The core problem of early development is that of *regional specification*, also called *pattern formation* or *spatial organisation*. This refers to the process whereby cells in different regions of the embryo become switched onto different pathways of development. It is the mechanism of this process with which we are primarily concerned in this book. Regional specification should not be confused either with *cell differentiation* or with *cell movement*, which are processes posing us with important but distinct problems. Cell differentiation nowadays means the synthesis of new species of protein – species which are different from those made by the cell itself or by its ancestors at previous times, and different from those made by other cells in the embryo at the same time. The movement of cells in the embryo is by no means understood but is presumably related to cell differentiation in the sense that a cell expressing a certain group of substances on its surface will interact with its neighbours and with the extracellular matrix in such a way as to migrate in a certain direction and

stop at a certain place. These processes are of immense importance and clearly without them there would be no development. However, they are not extensively discussed here because they are consequences and not causes of regional specification. Of course once the first regional distinctions have been made the competence of cell populations to make further decisions will depend on the specific differentiation already achieved, but it is still possible to distinguish between the process of specification and its consequences.

The book itself is divided into three sections. Chapter 2 surveys the concepts of experimental embryology in an attempt to establish an unambiguous language for discussing the phenomena. Chapters 3 to 6 review what is known about regional specification in a variety of types of animal embryo. Chapters 7 to 10 ask what sort of mechanisms have been proposed to account for the phenomena and how well they stand up in the light of the evidence.

Universality and homology

For ethical reasons most experimental embryology relates to animals other than the human. Mammalian embryos are usually felt to be the best models for man but viviparity poses some serious technical difficulties and so most experimental embryology before the recent period has concerned itself with invertebrates and non-mammalian vertebrates. It is therefore an issue of some importance to know how similar are the mechanisms for regional specification in different types of animal. There are two ways of looking at this. On the one hand the universalist will say that the mechanism of inheritance, protein synthesis and cell ultrastructure are the same in all eukaryotic organisms so probably the mechanisms of early developmental decisions will be the same as well. On the other hand his opponent would argue that it is the formation of the basic body plan which is at stake here and different animal phyla are distinguished from one another precisely because they have different basic body plans, so we might expect their mechanisms of formation to be different.

Where different animals contain visible homologous parts which arise in homologous ways, it seems probable that the same biochemical mechanisms underly their formation. For example the neural tube arises from dorsal ectoderm in all vertebrates. In amphibians and chicks there is good evidence that it is formed as a result of a signal from the underlying mesoderm, and there is a little evidence that the same is also true in ascidians. It would be surprising if different mechanisms were at work in a case like this where not only is the final morphology the same but similar cellular interactions bring this morphology into being.

A similar argument can be made for the different species of insect, whose embryos can be seen to have the same basic structure at the late

germ band stage. Double posterior duplications (double abdomens) can arise in the fruit fly, in the midge and in the leafhopper as a result of various manipulations (see Chapter 4). The fact that the same striking abnormality in the body plan can arise in different species following experimental interference again argues for the existence of a common underlying mechanism.

But is there anything in common between insects and vertebrates? The basic body plans are completely different and the observable course of early development is also completely different. If there is a common mechanism for early developmental decisions it will presumably be one which was already in existence in the common metazoan ancestor of vertebrates and insects, and we do not know anything at all about such an ancestor, or even that there ever was one.

On the other hand there are a number of properties of the early developmental mechanisms which do seem to be similar not only between vertebrates and insects but between all the groups of animals considered in this book. These are not related to particular anatomical features but rather to behaviours in the face of experimental interference, one example being the universal ability of early embryos to regulate their proportions following an alteration in overall size. These properties are collected and discussed in Chapter 7, but whether the similarities are significant or merely fortuitous will not be known for certain until the mechanisms themselves are understood at a biochemical level. Perhaps the problem of universality is itself a deep enough question to justify the study of types of embryo far removed from man, although the exigencies of funding for medical research will inevitably continue to favour the vertebrates at the expense of the invertebrate phyla.

The developmental hierarchy

It is very important to emphasise that even the basic body plan is not specified all at once but is formed as a result of a hierarchy of developmental decisions. This statement will be justified more fully by the experimental results reviewed in Part II, but for the moment consider an embryological lineage such as that illustrated in Fig. 1.1. This shows the provenance of different regions of the vertebrate body deduced mainly from the experimental embryology of the Amphibia. The diagram comprises a number of subdivisions of different multicellular regions. Without considering the mechanism of these decisions, which is the subject of the remainder of the book, we note that the familiar histological cell types are to be found only on the right side of the diagram. Each decision is irreversible and so all the states which precede terminal differentiation each embody a reduction of potency compared with the previous state. For example, for cells to be competent to form the lens of the eye they

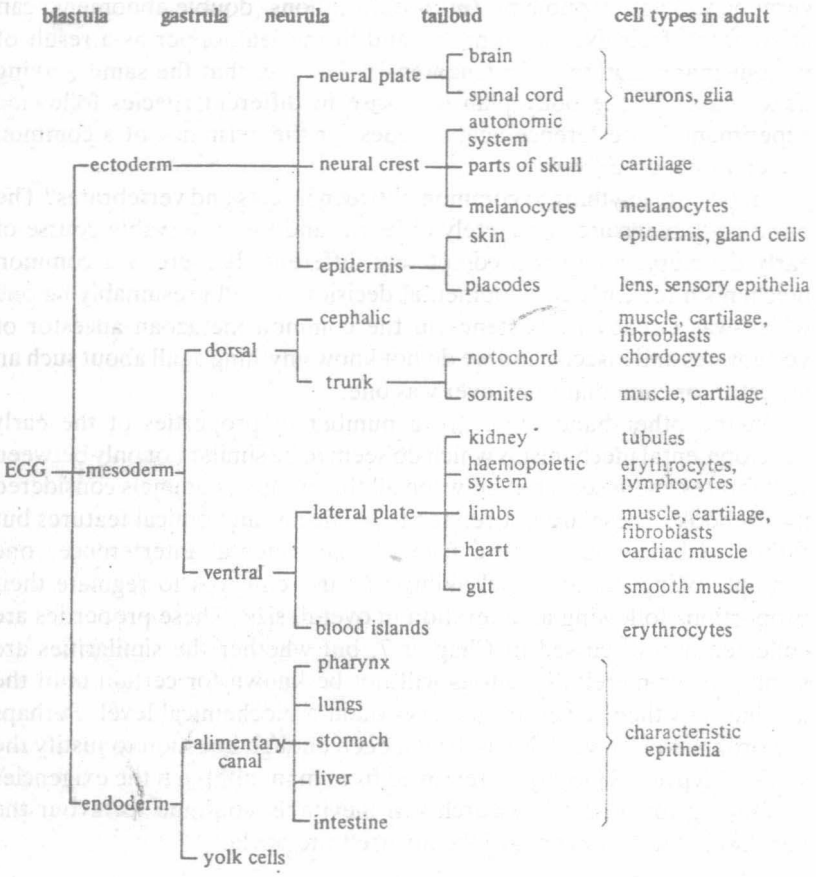

Fig. 1.1. Formation of the basic body plan in a vertebrate (excluding extra-embryonic regions). By the early tailbud stage the embryo consists of a mosaic of regions determined to form the principal organs and structures of the body. This body plan is built up as a result of a hierarchy of decisions, and several further decisions will in most cases be taken before the cells differentiate into the terminal cell types shown on the right-hand side. It should be noted that some cell types, such as cartilage, arise from more than one lineage. Further details will be found in Chapters 3 and 6.

must have already decided that they are ectoderm (rather than mesoderm or endoderm), and epidermis (rather than neural plate or neural crest). So each decision is made by cells in a particular determined state, it is made among a small number of alternatives and the outcomes are not in general terminally differentiated cell types but new states of determination whose potency is further restricted. It is because of the conviction that the basic body plan arises from a *hierarchy* of decisions between determined states that this book is subtitled 'determinative events in early

development'. Unfortunately, with the exception of the three 'germ layers', whose existence was deduced in the nineteenth century from descriptive embryology, there are no suitable names for the determined states. We are thus driven to referring either to positions ('anterior mesoderm', 'posterior mesoderm') or to the future organ ('limb field', 'eye field'), and this makes embryological terminology rather obscure and confusing to the outsider.

Only two general processes are known which account for the events of regional subdivision: *cytoplasmic localisation* and *induction*. We do not understand either very well and in no case do we know the biochemical basis of the phenomena. Cytoplasmic localisation has been studied mainly in invertebrate embryos with a small total cell number (see Chapter 5). It appears that regulatory molecules, whose identity is unknown, become differentially distributed in the cytoplasm of a stem cell. In the course of an asymmetrical cell division the two daughter cells inherit different materials and consequently enter different states of determination.

Induction has been studied mainly in vertebrate and insect embryos. Here it appears that the competent tissue becomes differentially determined in response to chemical signals from other regions of the embryo. The possible mechanisms for induction are extensively discussed in Chapters 9 and 10.

The distinction between cytoplasmic localisation and induction is one which has been made using classical techniques of experimental embryology and it is clear that both processes are widespread and probably occur in all types of embryo. Unfortunately the classical techniques are limited when it comes to analysing further the mechanisms of these processes and this becomes particularly obvious when we consider the near impossibility of excluding various theoretical models which have been proposed in recent years. Obviously new experimental methods are needed, but exactly what they should be is not so clear.

Positional information and non-equivalence

The recent upsurge of interest in regional specification owes a good deal to the 'positional information' concept of Wolpert (1969, 1971). This is often confused with a specific model for induction, the diffusible morphogen model, which is discussed in Chapter 9. However, the doctrine does not involve belief in any particular model but rather a set of general precepts about embryonic development. The term 'positional information' has itself become rather ambiguous and is not used here, but the general precepts are listed since they have influenced the author in terms of the material selected for inclusion and the manner of its presentation:

1. Regional specification comes first, cell differentiation and cell movement are consequences.
2. Regional specification can always be broken down into two independent processes: an instructive process during which positional information is imparted, and an initial response by the competent tissue called 'interpretation'.
3. The biochemical mechanism underlying positional information is the same in all animals. The mechanism of interpretation differs according to the particular anatomy being formed.
4. Cells which end up with the same histological type, but which are of different embryological provenance, are at least transiently 'non-equivalent', i.e., exist in different states of determination.

Wolpertians will be able to read this book if they substitute 'positional signal' for 'inductive signal' and 'positional value' for 'state of determination'. The more traditional terms have been employed here both because they are more generally understood and because the Wolpertian terminology does not lend itself to situations involving a hierarchy of decisions which are typical of early embryos.

The concept of non-equivalence (Lewis & Wolpert, 1976) is particularly important because it is implicit in most modern thinking about regional specification and has far-reaching implications. The idea is that in an organ such as the limb, which consists of quasi-repetitive arrangements of muscles and skeletal elements, the different parts of the embryonic rudiment necessarily acquire *different* states of determination as a result of the regionalisation process, even though they may end up by becoming the same histological cell type. In connection with this it should be noted that the same cell type can arise by different routes in different parts of the embryo (Fig. 1.1). Evidence for non-equivalence can be found in the fact that the program of maturation may be visibly different: for example cartilages of the wrist grow less than those of the long bones (Summerbell, 1976) and different cartilages become ossified at different times (Holder, 1978). In cases of animals which are capable of extensive regeneration it seems probable that the information encoded in the states of determination persists into adult life and can be recalled following the removal of parts (Slack, 1980*a*).

The implications of non-equivalence are twofold. First, if this way of looking at things is correct, the process of regional specification is complex. As we shall see in Chapter 10, it can be shown that an organism consisting of n parts will need something more than n biochemical components (genes, switches, substances) to specify that they should develop in the correct relative positions. So an organism as complex as man would have an appreciable fraction of the genome devoted to controlling regional specification. This is an area of cellular activity about

which we presently know next to nothing at the biochemical level, and we can anticipate that its unravelling may take several decades of research activity by biochemists and molecular biologists.

Secondly, if non-equivalence is not transient but persists into adult life, then the organism must possess a 'second anatomy' of codings expressing embryological decisions, which is superimposed on the familiar visible anatomy. The implications of this are discussed elsewhere (Slack, 1982).

Since positional information and non-equivalence are inseparable concepts it follows that mechanisms which generate a series of structures in identical states of determination are not positional information mechanisms. Some of these will be discussed in Chapter 10 in connection with the determination of repeating structures such as segments and somites.

Molecular biology

The other major new influence on contemporary thinking in embryology comes from molecular biology and is, for example, well articulated by Davidson (1976). Molecular biologists argue that different sorts of cells exist because they are expressing different genes and therefore the control of gene expression is the central problem of developmental biology. Some progress has been made in recent years in identifying regulatory sequences which control gene expression in animal cells and as this is such an active area of research it is sure that the molecular details will soon be known in a few cases.

Unfortunately, for the embryologist it will not help very much to know how one or two particular genes are regulated. To understand the course of development it will be necessary to predict the pattern of gene activity in a cell from knowing the previous pattern of activity and the significant influences from the environment. This cannot be done without knowing what controls the activity of each and every gene. In bacteria we know that different genes are regulated by different substances and indeed that there are several basically different types of mechanism involved. The number of genes in animals is not really known but is usually estimated in the range 10^4–10^6 (see Chapter 8), so it will certainly take a great deal of time to build up enough knowledge of gene regulation to be able to explain, or predict, the course of embryonic development.

Despite rapid progress in molecular biology it must be admitted that it has yet to make a major contribution to our understanding of early development. This may be because some of the gene products involved in terminal differentiation are known while those involved in early determinative decisions are not. In the following chapters biochemical and genetic results are included and discussed where they are relevant, but the