Tertiary Level Biology

The Genetic Basis of Development

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Preface

This book discusses the significance of genetical concepts and techniques in developmental biology. It is aimed at students in their final undergraduate year, and at researchers at all levels who do not have genetical expertise and would like to consider its relevance to their work. We have assumed a previous understanding of eukaryotic genetics such as would be obtained from most broad introductory genetics courses. There is a strong emphasis on the description of genetic systems. Specific experiments are then evaluated in relation to the conceptual analysis of the developmental process which emerges as the book proceeds.

Most books are as notable for what they leave out as for what they include. In choosing the experiments, we have tried to keep a balance between conflicting requirements—for example, to consider the variety of different organisms, whilst ensuring that there is sufficient continuity from one chapter to the next for a picture of different developmental strategies to be constructed. In practice, this has meant that there is a stronger reliance on animals—*Drosophila* and the mouse in particular—than might otherwise be desirable. We feel that this is inevitable at the moment, given the amount of work done on these species. Chapter 4 (on the molecular biology of eukaryotic cells) is deliberately limited, as this subject is complex and is itself developing very rapidly. Here we have aimed merely to provide background information for other chapters, and to show that the use of gene cloning and sequencing techniques is likely to revolutionize our understanding of the organization of the genome and the regulation of gene expression. The final chapter considers one topic—sexual differentiation of mammals—in greater depth, and serves to illustrate the interrelationships between mechanisms acting at the different levels of organization which have been discussed separately in preceding chapters.

Several excellent books on developmental biology are now available, some of which use genetical examples. Although the regulation of gene expression is generally accepted as the basic paradigm for the understanding of development, many texts rely almost exclusively on the analysis of non-genetical studies to provide a bridge between the genome and the phenotype (the organism). This book gives expression to a growing awareness that a multi-disciplinary approach between geneticists, biochemists, developmental biologists and others is of crucial importance, and expounds the particular contribution of genetics in the context of the understanding provided by other disciplines. It is intended to complement the more traditional books on developmental biology, and can also be read in conjunction with books on eukaryotic molecular biology.

We would like to thank the friends, colleagues and students, too numerous to name, who have discussed these ideas with us. We are very grateful to Dr S. Baumberg for his helpful comments on a draft of chapter 4, and would like to thank Mrs Betty Sharp, Mrs Audrey Stewart, Mrs Doreen Jobbins and Mrs Carol Cusworth for typing. We are indebted to Mrs Joan Stratford for the production of the final manuscript.

In any book of this kind, there are bound to be some errors in description, and differences of opinion concerning the interpretation of experimental data. We would welcome any comments readers may wish to send to us.

A.S.
D.H.

Illustrations drawn by Terry Collins, Leeds Polytechnic

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CHAPTER ONE

INTRODUCTION

This book is concerned with the processes of development in multicellular organisms. From before the turn of the century, clear descriptions have existed of the different stages in the development of many species of plants and animals, of their different life cycles, and of the role of cell movement in morphogenesis. In an effort to understand the mechanisms involved, many studies utilizing techniques for the experimental manipulation of embryogenesis have been carried out. The importance of the nucleus in development was clearly revealed in experiments such as those undertaken by Boveri and Hämmerling. Boveri enucleated sea urchin eggs of one species before artificially fertilizing them with sperm from a related species differing in features such as pigmentation and the form of the pluteus larva. For these characters, the artificial hybrids developed according to the nucleus rather than the egg, indicating that the nucleus rather than the cytoplasm directs morphogenesis. Hämmerling also examined the respective roles of the nucleus and cytoplasm in the unicellular alga, *Acetabularia*. He grafted the stem of one species on to the rhizome (which retained the nucleus) of a different species (figure 1.1). A new cap was formed, intermediate in appearance between those of the two parental types. If this was cut off, a second cap regenerated, whose shape resembled that of the species which donated the nucleus, rather than the one which provided the bulk of the cytoplasm (as the stem of the hybrid).

The concept that the roles of cytoplasm and nucleus should be seen as an interaction between the two components of the egg, rather than one or the other representing a decisive influence in isolation, emerges clearly from a classical experiment carried out on the egg of the dragonfly *Platycnemis* by Seidel in 1929. When he made a constriction with a hair loop at the posterior end of the egg before cleavage nuclei had time to interact with

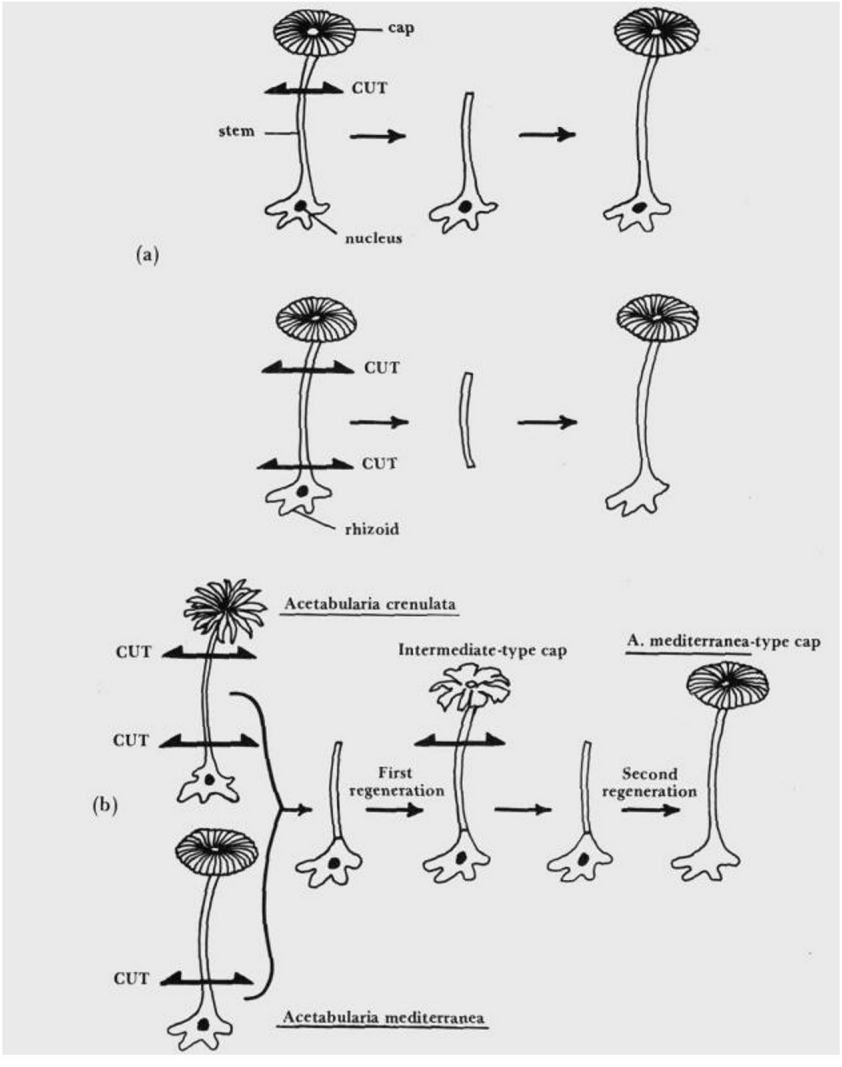

Figure 1.1 Hämmerling's grafting experiments with *Acetabularia* species: (a) removal of cap results in regeneration of a new cap whether a nucleus is present or not (b) rhizoid with a nucleus from *A. mediterranea* grafted to a stem of *A. crenulata* eventually produces an *A. mediterranea* type of cap.

this region, no embryo was produced from the anterior end. Alternatively, if the ligature was not pulled completely tight so that some cleavage nuclei entered this region, or if the constriction was made after the nuclei had

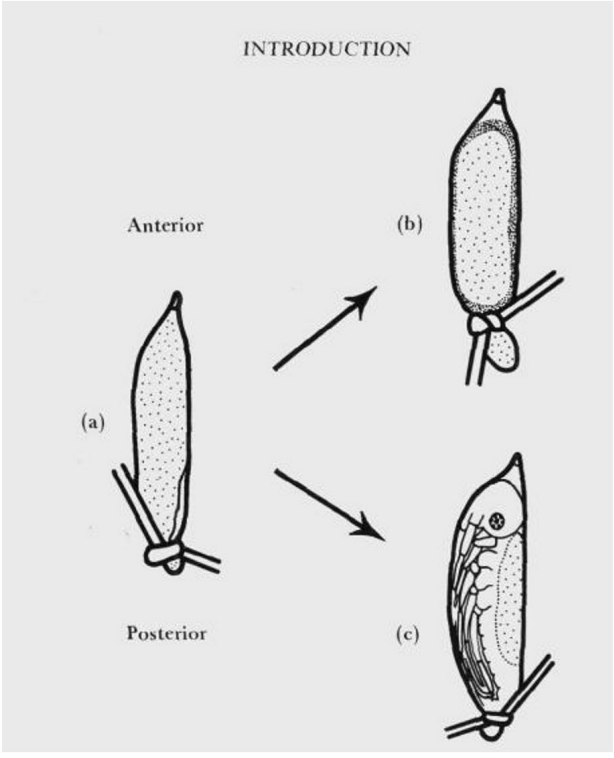

Figure 1.2 Seidel's experiments with the dragonfly, *Platycnemis*: (a) The posterior region of the egg is constricted; (b) With early constriction, the embryo fails to develop; (c) With late constriction, when any of the 128 blastoderm nuclei has passed into the posterior region, a complete embryo is formed.

entered and interacted with the posterior cytoplasm, development of the embryo proceeded normally (figure 1.2). From this it is apparent that a particular region of the peripheral cytoplasm at the posterior pole (the *activation centre*) is necessary to initiate embryogenesis, and that it can act only when a nucleus migrates into it. In fact, Seidel was able to show that any of the 128 nuclei present at the migratory phase of development were capable of mediating the action of this region.

Although these elegant experiments clearly demonstrated the importance of the nucleus in development, its mode of action remained almost completely unknown. Many experimental embryologists confined their attention to defining the role of cytoplasmic determinants and cell interactions in the control of developmental processes. However, following the rediscovery of Mendel's work at the beginning of the century, the science of genetics developed rapidly. Primary interest concerned the mode

of gene action and the nature of the gene; but geneticists were also aware of the developmental anomalies produced by the mutations that they studied. The idea that development proceeded by the controlled switching on and off of particular gene loci in a sequence characteristic to each tissue was developed in the 1920's. Progress in the application of genetic techniques to the study of development owes much to the influence of R. Goldschmidt and C. H. Waddington. In his book *The Principles of Embryology* (1956), Waddington emphasized the genetic control of developmental pathways whilst giving a masterly summary of the findings of experimental embryology. Even so, these two aspects had largely to be presented separately. Among the reasons for this were the unknown nature of cytoplasmic determinants and the obscure mechanisms of tissue interactions on the one hand, and the inadequate models of the mode of gene action on the other. This dichotomy still persists. In many introductory texts, the regulation of gene expression is cited as the fundamental mechanism in development, but thereafter genetic studies are barely referred to. Nevertheless, genetic variation can be found affecting every phase of development. The aim of this book is to show how the manipulation of the genome has been combined with the experimental methods of biochemistry, endocrinology, physiology, immunology and classical embryology to provide new insights into the control of developmental processes.

The next two chapters examine the evidence for the constancy of the genome in development and the complementary studies on the production and role of cytoplasmic determinants in the egg. The following chapters look at the mechanisms of control of gene expression in eukaryotes. This is an area that has benefited considerably from the application of the new techniques of gene cloning and restriction enzyme analysis. The essential features of these techniques are reviewed and their application to the study of developmental regulation is examined. The organization of developmental processes in the embryo is then considered—whether developmental programmes are autonomous to a cell or group of cells in a tissue, or whether they depend on interactions with other cells or tissues. The interaction of cells is also discussed from a mechanistic standpoint. Finally, the book considers one complex developmental system where these processes can be seen in relation to each other.

CHAPTER TWO

THE CONSTANCY OF THE GENOME

Differential gene action during development could be achieved by two basic mechanisms. All cells might receive a complement of genetic information identical to that of the original fertilized egg. Different sets of gene products could then be formed if different regions of this constant genome are transcribed into RNA in different cells, or if there are differences in the processing of the transcribed RNA into mRNA, in its translation into protein, or in the activation (or degradation) of the protein. Alternatively, the various cells could receive different genetic complements, involving either the loss of particular gene loci or of whole chromosomes, or the formation of multiple copies of loci or chromosomes. This process would itself have to be controlled in the different cell lines, giving rise to a number of different clones of cells. Differential gene expression could also occur within the various clones. This chapter discusses the experimental evidence for the constancy of the genome.

Experimentally, various tests have been devised to distinguish between the mechanisms of differential gene expression and chromosomal differentiation. Broadly speaking, these tests either seek to examine the chromosome complements of differentiated cells by cytological or biochemical means, or to establish whether a differentiated cell or nucleus still retains the genetic ability to participate in the differentiation of another cell-type (a functional test of pluripotency) or even another whole organism (a functional test of totipotency).

Karyotypes

In the great majority of cases, mitosis during development results in differentiated cells with nuclei containing the same number of chromo-

somes (visible only during cell division) with identical morphology in the different tissues when viewed with the light microscope. Likewise, the DNA content per cell of different tissues is usually identical within experimental error. There are also exceptional cells which contain only half

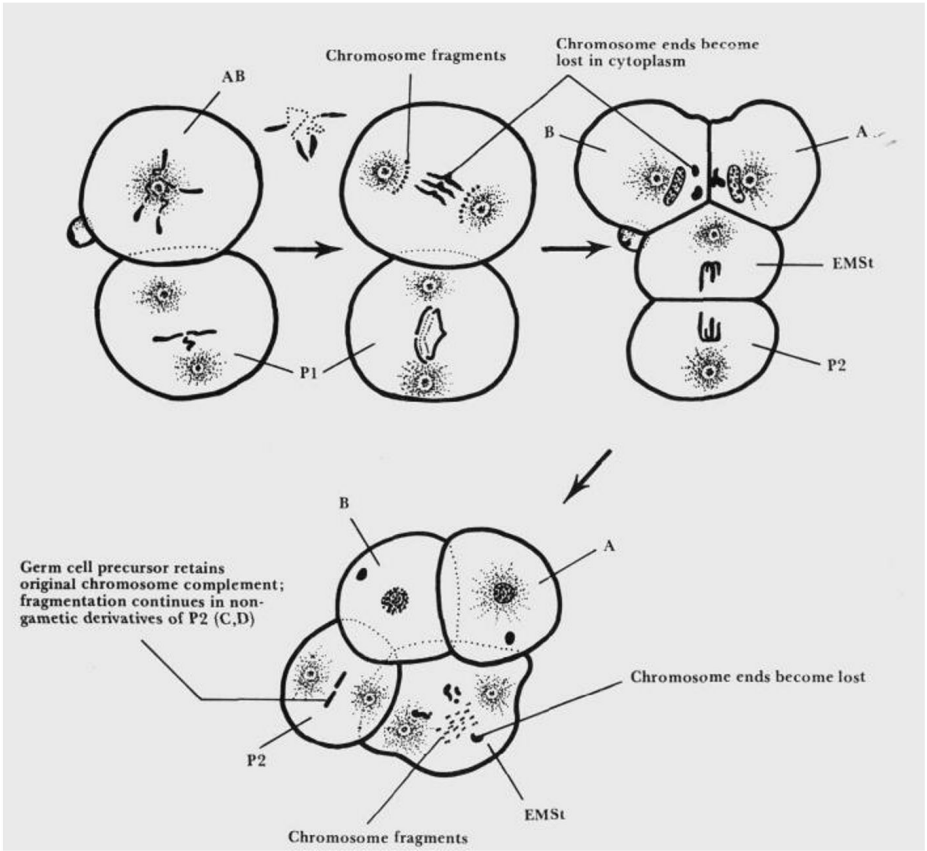

Figure 2.1 Cell lineage: dorsal cell family (AB) → most of ectoderm; EMSt → endoderm, anterior mesoderm, rudiment of stomadeum;

$$P_2 \rightarrow P_3 \rightarrow P_4 \rightarrow \text{germ cells}$$
$$\downarrow$$
$$D \rightarrow \text{posterior mesoderm}$$
$$\downarrow$$
$$C \rightarrow \text{posterior ectoderm}$$

Chromosome fragmentation in the early development of the nematode, *Ascaris* sp., as described by Boveri in 1899.

the number of chromosomes and DNA (such as spermatozoa), cells which lose their nucleus when differentiated (mammalian erythrocytes and plant xylem cells) and cells which are multinucleate or polyploid (mammalian myoblasts and liver, tobacco pith and plant roots). These changes, although a feature of the differentiation of those cells, cannot in themselves result in the formation of different gene products in different cells as they affect all loci equally.

In some cases, differential distribution of chromosomes is seen early in development. In nematode worms such as *Ascaris*, the cell line which gives rise to the germ cells and the gametes retains the full chromosome complement (by definition), whereas fragmentation and loss of satellite DNA sequences (see p. 13) from the ends of the chromosomes (Moritz and Roth, 1976) occurs in the other cell-lines in successive cell divisions (figure 2.1). The reduced chromosomal complement in the somatic tissues apparently remains stable during subsequent development. However, the functional consequences of the differences in karyotype between germ cells and somatic tissues are not understood, and the differences between the various somatic tissues cannot be attributed to the differential chromosome loss. In experiments where the egg of *Ascaris* was subjected to centrifugation in order to redistribute the cytoplasm, it was found that components of the cytoplasm localized at the vegetal pole protect the nuclei from chromosome loss and diminution. A similar phenomenon is seen in the gall midge *Wachtiella persicariae* (Geyer-Duszynska, 1959), and in the fungus gnat *Sciara coprophila* (Crouse, 1960) where certain entire chromosomes of the full complement are lost in somatic tissues only, and in the development of the *Sorghum* plant (Darlington and Thomas, 1941) where the supernumerary *B* chromosomes (which do not pair at meiosis) are lost from the root tissues, but are retained in some shoot tissues, and are transmitted to the next generation by the gametes formed in the flowers. However, these few cases of variation in the karyotype of different tissues are exceptions to the general rule of regular mitosis in plant and animal development.

Polytene chromosomes

In the Diptera, unusually large chromosomes, called polytene chromosomes, can be seen with the light microscope even during interphase. These occur in various tissues, such as the salivary gland, Malpighian tubule, rectum, mid-gut, oesophagus, tracheal wall and muscle of the larva, the bristle socket and footpad epidermis of the pupa, and Malpighian tubules

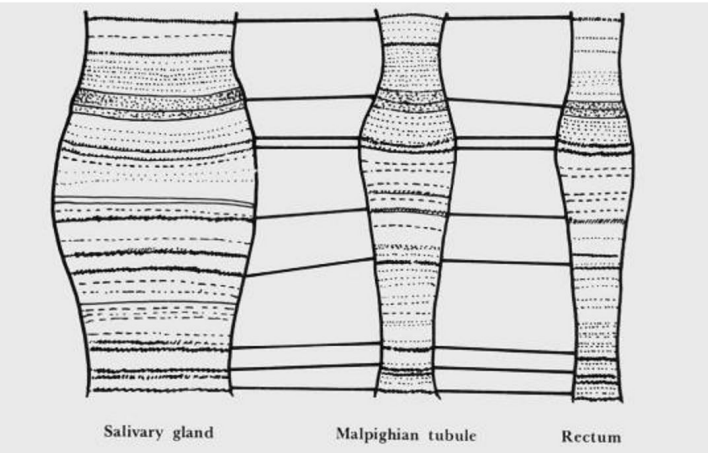

Figure 2.2 Identification of homologous bands in polytene chromosome III of *Chironomus* (Redrawn from Fischberg and Blackler).

and ovarian nurse cells of the adult (figure 2.2). Polytene chromosomes arise by the process of endomitosis whereby chromosome replication proceeds in the absence of strand separation and cell division. The homologous chromosomes are initially paired and remain so during replication. Polyteny therefore provides a mechanism for the production of multiple gene copies in the cell, permitting a high rate of synthesis of particular gene products. Unpaired polytene chromosomes have been seen in some species of other insects (e.g. *Collembola*), in ciliates, and in the bean (*Phaseolus*), but the dipteran salivary gland chromosomes have been the most intensively studied. The individual chromatid strands of the chromosome are in a greatly extended state (compared to a normal mitotic chromosome) and are all precisely longitudinally aligned. When *Drosophila* chromosomes are stained with suitable dyes, about two thousand characteristic transverse bands or chromomeres can be seen with the light microscope. As this number corresponds approximately with the estimated number of structural loci coding for enzymes and other polypeptide chains in these insects, it has been suggested that each boundary between a band and an interband may contain a structural locus for a protein. More precisely, Judd and colleagues (1972) have estimated the number of genes in a small region of the X chromosome containing a total of 15 chromomeres. By isolating a large number of recessive lethal mutations mapping in this region, they were able to identify 16 comple-

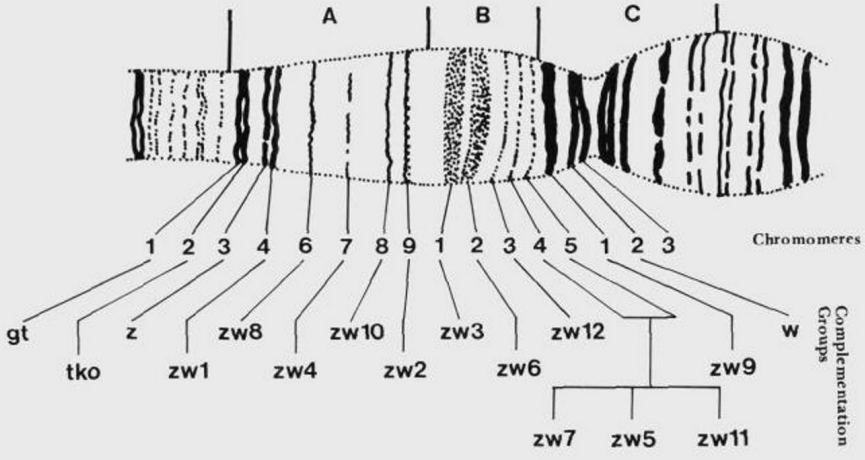

Figure 2.3 Assignment of complementation groups to chromomeres in the 3A–3C2 region of the X-chromosome of *Drosophila melanogaster*. The complementation groups are identified by the isolation of recessive lethals followed by complementation tests, and assigned to chromomeres by complementation tests with deletions which remove one or more bands from this region. (From Judd *et al.*, 1972).

mentation groups or genes. The analysis was subsequently extended to include non-lethal mutants (Judd and Young, 1973), and although additional genes were identified it is still probable that each chromomere contains either a single gene or at most a few loci (figure 2.3).

With normal karyotyping procedures, functionally significant chromosomal deletions or duplications could occur during development and yet escape detection. Although polytene chromosomes represent a nuclear specialization similar to polyploidy or multi-nucleation, they have been used to examine the possible occurrence of differential chromosomal changes during development with a much higher degree of resolution. Beerman (1952) carried out a comparison between different tissues of the banding patterns in chromosome 3 of the midge *Chironomus tentans* and found that he could identify the same bands in salivary glands, Malpighian tubules, rectum and mid-gut (figure 2.2). This and similar studies have been taken to provide strong evidence for the constancy of the genome.

Recently, though, Ribbert (1979) has reported that the banding patterns of polytene chromosomes from different tissues in the blowfly *Calliphora erythrocephala* bear little resemblance to each other, so that it may be necessary to revise this interpretation. The bands of this organism could be interpreted as reflections of functional activity, rather than as structural