

## Introduction: Towards a molecular analysis of development

During embryogenesis a multicellular organism emerges from a single cell which divides a finite number of times, in a spatially and temporally ordered way, to generate an ensemble of different cells (Fig. 1.1). The aim of developmental biology is to understand how this occurs. What are the elements that define the different kinds of cells? Where do the instructions for the process lie? What is the language of those instructions?

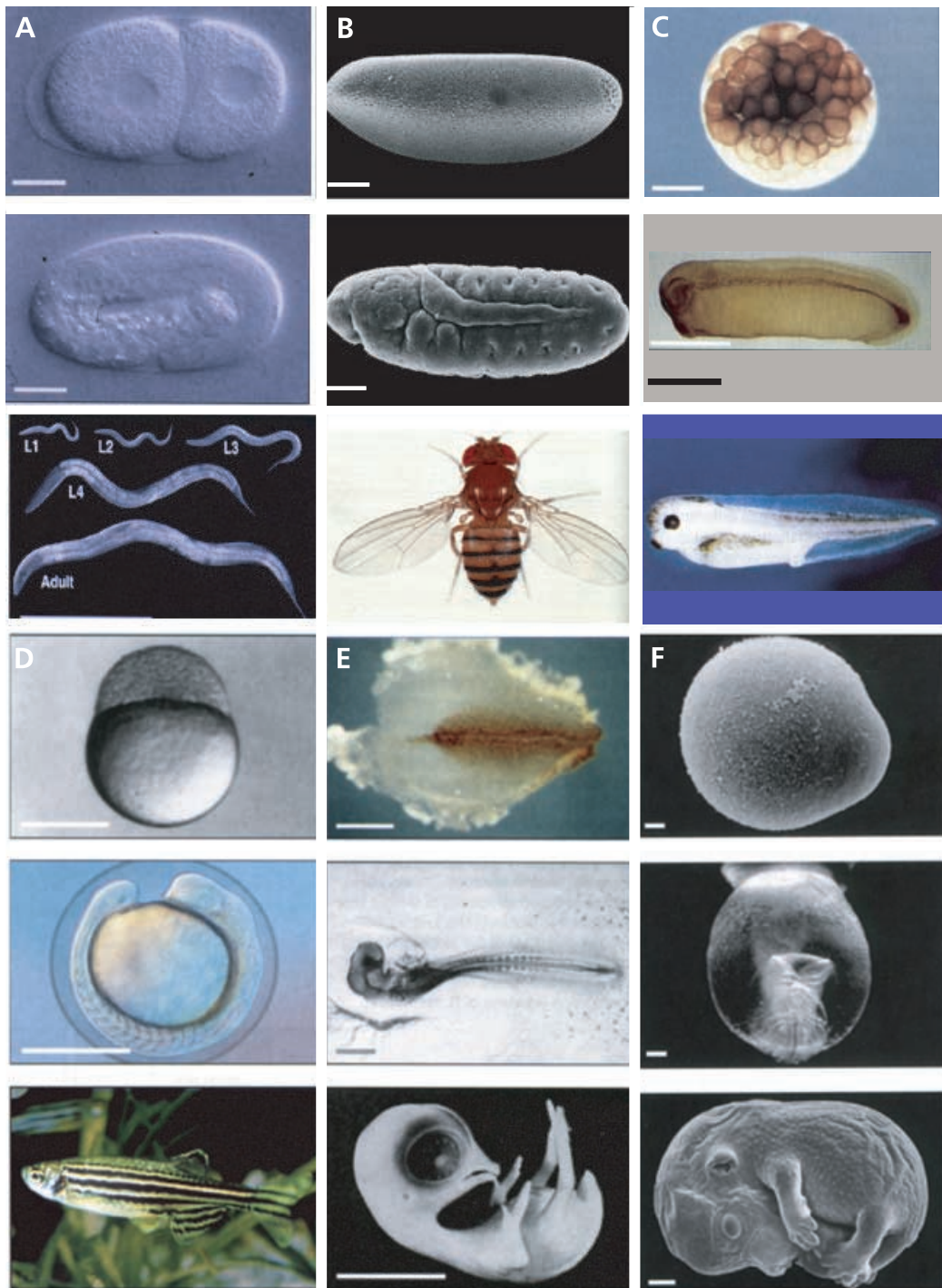
Classical approaches to these questions are based on describing the embryonic development of various animals. Moving to a finer level of resolution, they emphasize the central role that cells play in development, describe the emergence of complexity in different organisms in terms of what cells are doing, and discuss the strategies used by groups of cells to guide and coordinate developmental processes. Implicit in this approach is the idea that many experimental observations can be explained by the activities of specialized groups of cells, such as ‘organizers’, which can instruct the development of other cells. In more recent years, as the genetic and molecular mechanisms underlying cellular behaviour have begun to be elucidated, this molecular information has been added to the analysis.

Here we shall approach development from a different standpoint, one in which the molecules and the genes that encode them, rather than the organism, take centre stage. We do this in the belief that the logic of development can only be appreciated by beginning with the genetic programs that underlie development. These programs both encode and are executed by molecular networks of proteins operating within and between cells. The protein networks create cells and direct basic cellular behaviours, or

‘routines’, such as cell adhesion, division or movement, which are deployed during the development of the organized cellular assemblies that make up tissues, organs and ultimately whole animals (Fig. 1.2).

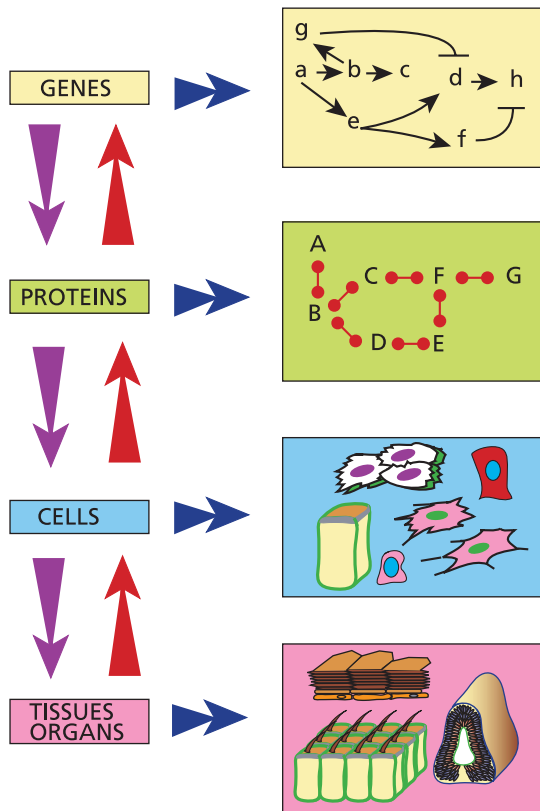
This analysis does not, however, imply a simple linear transformation of genetic information into shape and size, mediated by proteins, but a progressive unfolding of information to create different levels of complexity and organization (Fig. 1.2). The unfolding is based on the activities of a small number of functional modules, organized hierarchically with increasing levels of complexity. The components of a particular module (proteins, cells, tissues) are specified by the module in the level below, but its basic organization and properties depend exclusively on its own elements and cannot be predicted from those of the module below. For example, the properties of cellular ensembles or protein networks cannot be predicted from the genetic circuits that determine their composition (Fig. 1.2).

The different modules are linked through functional relationships that enable the developmental events they mediate to function smoothly and in a regulated way (Fig. 1.3). Thus, genes and protein networks interact to create regulatory circuits that integrate the activity of these two levels. Each level lays down the molecular prerequisites for interpreting the information that instructs the next level; in turn, this level will generate a new level of information processing and will modify the one that gave rise to it. This modification may lead to a new configuration of the whole system, advancing the developmental process. For example, a new set of proteins within a cell can change its pattern of gene expression, which may determine a transformation towards a new cell type.



**Fig. 1.1. The development of animal model systems.** Three different stages of development of various animals used as developmental model systems are depicted from top to bottom in each panel. Sizes of scale bars are indicated in the legend. **(A)** Nematode *Caenorhabditis elegans*. Two-cell embryo (10 $\mu$ m); larva (10 $\mu$ m); various larval stages with the adult worm. **(B)** Fruit fly *Drosophila melanogaster*. Blastoderm stage (0.1mm); extended germ band stage (0.1mm); adult fly. **(C)** Frog *Xenopus laevis*. Blastula stage

(0.5mm); tail bud stage (1mm); tadpole. **(D)** Zebrafish. Sphere stage with embryo on top (0.5mm); 14 somite stage displaying the basic body plan with head to the left (0.5mm); adult fish. **(E)** Chick. Gastrulating embryo (1mm); 2.5 days after laying (1mm); 8.5–9 days after laying. **(F)** Mouse. Zygote before the first cleavage (10 $\mu$ m); 8 days after fertilization (0.1mm); 14 days after fertilization. Images from Wolpert, L. (1998) *Principles of development*, Current Biology and Oxford University Press.



It follows from this view of development that it is misleading to think of the genome as embodying the ‘blueprint’ of an organism. This often-heard architectural analogy implies that the DNA sequence of an organism contains, in a more or less straightforward way, both the information for the materials to make an organism and the instructions for its assembly. The sequence of the genome contains some of the information to build an organism but this information is very limited. Genes encode proteins and carry information that will determine when and where these proteins are made. However, genes do not contain the information that determines how the proteins will assemble, or when, where and how will they be active. These pieces of information, essential in any ‘developmental blueprint’, lie in the proteins themselves, which assemble into functional and regulatory networks following instructions embodied in their three-dimensional structures (Fig. 1.2). The constituent proteins of these networks contribute to the composition and activity of cells, determining and modulating their movement, shape, information-processing ability, and patterns of division and differentiation. At the same time, the activity of the net-

**Fig. 1.2. The hierarchical organization of biological systems.**

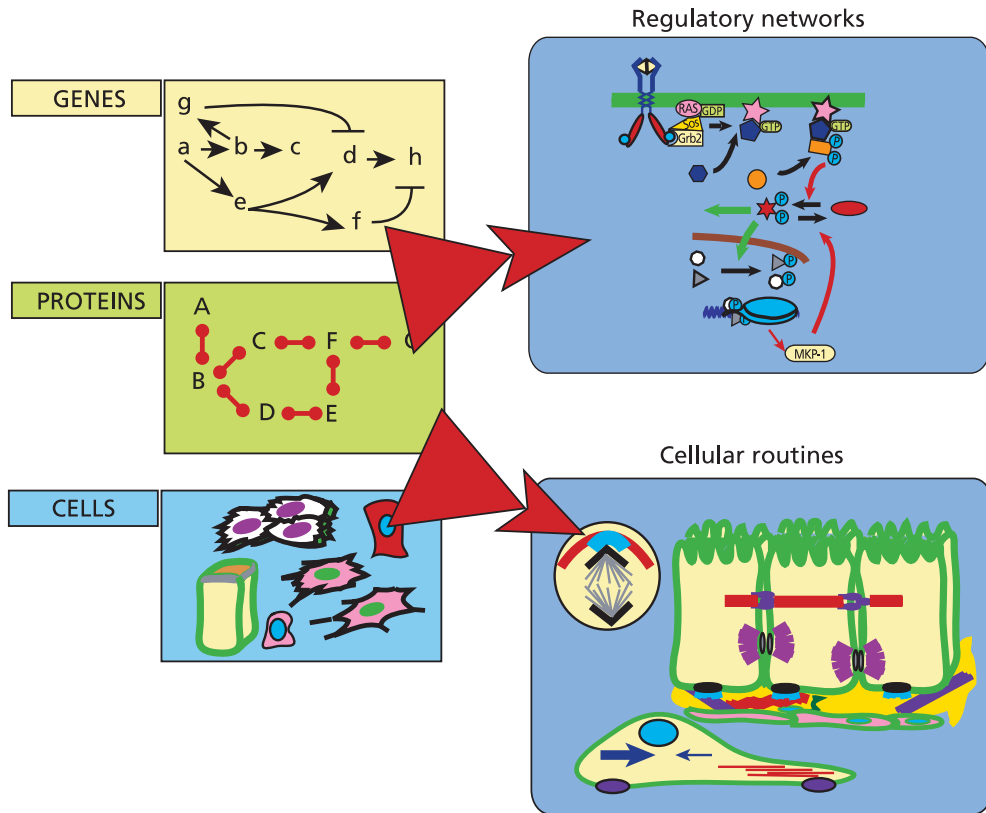
Biological systems are organized into functional modules of different levels of complexity, which are related through the activities of their component elements. An important rule of this organization is that a particular level provides the elements and information to generate the next higher level of complexity. Genes (a, b, c . . .) encode proteins (A, B, C . . .) which organize themselves into functional networks. Some of these functional networks act back on the genes to create regulatory relationships that generate networks of gene activity. Other protein networks assemble macromolecular structures which generate cells. The different appearances or functional attributes of cells arise from their different protein composition. In the same way that proteins have properties that cannot be predicted from the structure or functioning of genes, proteins work together in complexes within the context of cells in ways that transcend the properties of individual proteins. Interactions between cells can affect the operation of the protein networks and, in this way, influence gene activity. Cells assemble themselves into tissues and organs which develop properties of their own that are not easy to predict from the properties of their individual cellular components. At the next level, tissues and organs provide the structural basis of an organism.

works feeds information back to the genes that encode their elements, to create regulatory circuits of gene expression (Fig. 1.2, 1.3). In the same way that proteins can be said to be the creation of the genetic machinery, cells are the creation of the interaction between genes and proteins. Different combinations of these elements generate different cell types which will assemble into tissues and contribute to the shape and size of an organism (Fig. 1.2).

A better analogy for encapsulating the logic of development is a computer. The molecular networks can be thought of as the ‘hardware’ of developmental systems—a hardware made up of proteins that read and interpret different ‘programs’ to produce different outputs: different animals, different organs, and supracellular aggregates. The programs are written in the regulatory regions of the genome and determine the sequence of patterns of gene expression characteristic of each organism. This also means that the hardware that reads and interprets the programs is itself determined by gene expression programs.

## The rationale for a different approach

The impetus for a change in approach to development has come from the explosion of information over the last twenty years as the techniques for isolating and

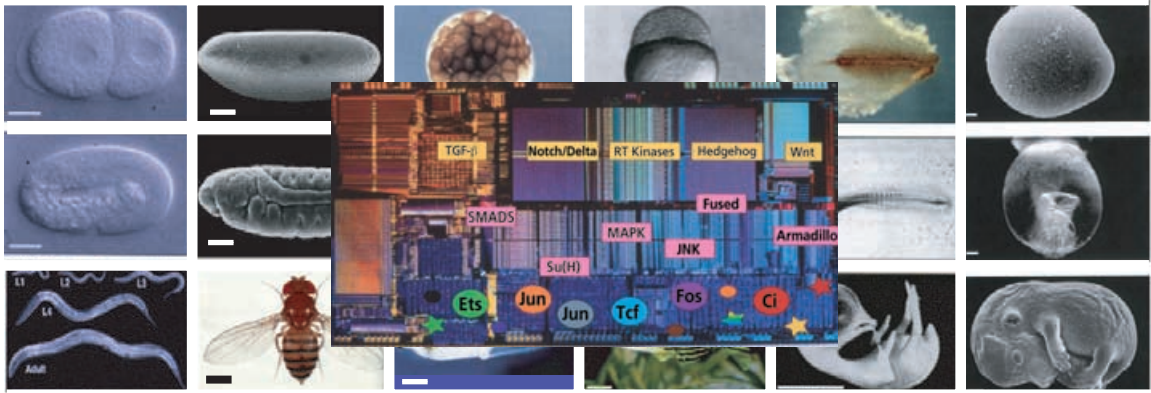


characterizing genes have been applied to the analysis of developmental processes. We now have an impressive and ever-growing list of genes and associated regulatory sequences involved in generating different cell types. We have realized that in order to understand developmental processes we need to understand the underlying genetic circuitry, that is, how the activity of one gene leads to the activity of other genes that are under its control and how these activities are modulated in space and time. We have also discovered that embryonic development and its component patterning processes rely on the activity of information-processing networks that act within and between cells. These networks are made up of proteins that function in the emission, reception, and transduction of chemical signals, and others that act as targets and effectors for these signals.

Perhaps the only justification that is really needed for moving aside from the organism-based approach to development is to point out the universal nature of these molecular mechanisms and networks. The studies of the last few years have shown that there are relatively few different

**Fig. 1.3. Assembly of the components of each level into functional modules.** Interactions between the different levels depicted in Fig. 1.2 generate functional modules that contribute to the building of an organism. For example, genes and proteins interact to organize regulatory networks that govern the patterns of gene activity of different cells. These patterns, in turn, determine the protein composition of those cells. The networks are usually represented as flow diagrams of interactions between different proteins and between proteins and genes. Similarly, proteins and the cells to which they contribute interact to generate cellular routines (such as adhesion, division or motility) that will participate in the construction and modulation of the large-scale cell assemblies seen in organs and tissues.

types of networks, that they are evolutionarily conserved, and that they are used both in different organisms and in different parts of the same organism. Looking at different developmental processes within the same organism, or even at the same process in different organisms, always reveals the involvement of the same signalling networks



and, sometimes, related patterns of nuclear activity. What this observation suggests is that the networks act as molecular processors of cellular information (Fig. 1.4); the output from these processors is determined by the protein composition and the genetic state of the cells on which they act. The same signal input will be processed differently in, for example, a neuron or a muscle cell, because one has a ‘neural’ genetic and molecular environment, while the other has a ‘myogenic’ one.

This principle helps to explain otherwise puzzling observations such as the appearance of extra limbs when a piece of embryonic head from which the ear will develop is implanted on the flank of an amphibian embryo (Fig. 1.5). This bizarre outcome of a strange experiment was only recently explained by the discovery that the initial stages in limb development require a signal encoded by the molecule fibroblast growth factor (FGF) and that the cells that will give rise to the ear, at a certain stage of development, are a source of this growth factor. Thus, if FGF acts on cells of the head it promotes the development of anterior structures, but if released on the flank of the embryo at the appropriate time, it will trigger the development of a limb. This is because the same signal is interpreted in different ways by cells in different states.

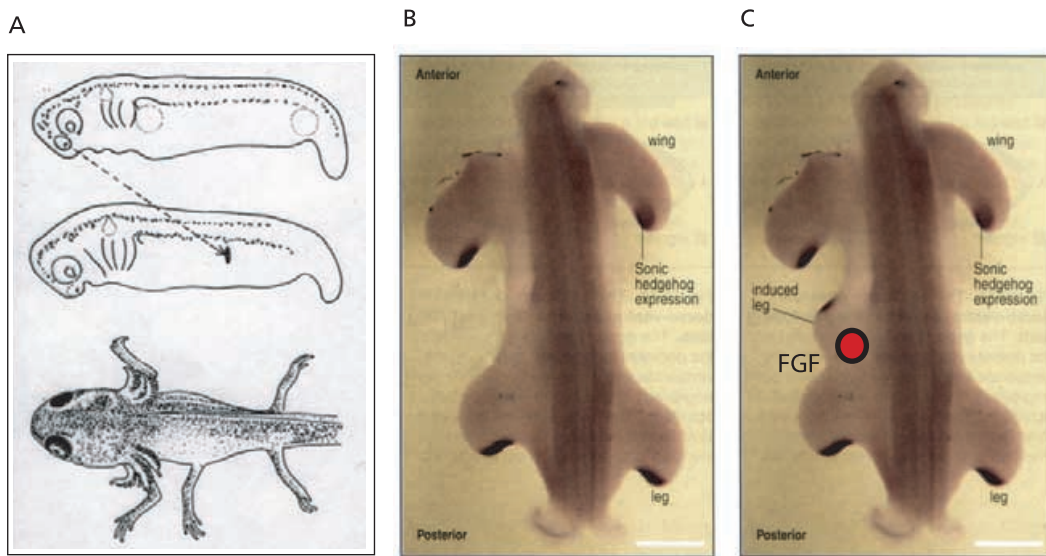
Returning to the analogy of the computer: the hardware of the cells in the anterior head of the embryo is very similar to the hardware in the flank but they are plugged into different programs and that is why the same signal, FGF, is interpreted in such different ways as a signal to follow the developmental pathway of either an ear or a limb. The output is not specified by the signal but by the state of the cell it acts upon. This state is measured by the presence or absence of an active network capable of interpreting the signal, and its constellation of active genes. In many other

**Fig. 1.4. The universal cellular microprocessor.** Despite the diversity in modes of development that characterizes the animal kingdom, there is an underlying theme of molecular unity. All organisms have a similar set of proteins that can be organized into classes. These proteins are able to process information and act as elements of a microprocessor. An interesting property of this microprocessor is that it can be transferred from one organism to another and will process information according to where it is placed. If an element of one organism (e.g. in the form of a gene encoding a protein) is placed in another, the gene will be expressed, the protein made and incorporated into the routines of the host, performing the tasks and contributing to the development of the host in a host-specific manner. The background images show, from left to right, embryos of *C. elegans*, *Drosophila*, *Xenopus*, zebrafish, chick and mouse. (Images from Wolpert, L. (1998) *Principles of development*, Current Biology and Oxford University Press.

cells of the embryo, FGF has no effect, that is, the signal cannot be interpreted or read.

A clear implication of this universal nature of the molecular networks is that, when trying to explain the mechanisms that control and execute developmental events, there might be only limited usefulness in talking in terms of model systems, whether whole organisms (e.g. the fish, the fly, the mouse) or specific organs or structures (the heart, the lung, the gut). Questions such as how to make a fly or a worm or a mouse are certainly interesting but before we can begin to set down the sequences of molecular events that underlie these processes, we need first to understand what different organisms have in common in terms of their information processing devices.

A basis for this understanding lies in appreciating the difference between mechanisms and strategies. In the context of developmental biology, mechanisms depend on the



molecules that encode and execute the various functions of a cell and are, probably, finite and conserved. On the other hand, strategies are the uses that cells make of the mechanisms to generate animals, and are vastly more varied. How the activation of a cell surface receptor leads to the expression of a particular gene or to cell division, or how the transcription of sets of genes is coordinately regulated, are examples of mechanisms. The way a cell or a group of cells uses these to generate particular spatial patterns is an example of a strategy. In the example of the activities of FGF, the mechanism of action of FGF is the same in the head or in the flank of the embryo, in a chick or in a sheep. How this basic mechanism is used in the development of a particular organ or an animal, by controlling when and where the signal is released or whether the signalling system is operative, are examples of strategies.

In this book, our goal is to establish a basis for dealing with the mechanisms that lead cells to become different within groups, to organize themselves in space and time, and to give rise to the patterns that make up organisms. Because the molecular elements that govern cellular activities are conserved at some basic level it seems reasonable to assume that the problem does not require a solution for every particular case, but that each system might have something to offer that can be extrapolated to a general framework. For this reason the book will always address the issues from the point of view of the mechanism and not of the system or organism.

For the same reason, we break with the classical idea that precise descriptions of developing embryos are needed in

**Fig. 1.5. Multiple functionality of single signals.** (A) If a piece of tissue from the anterior region of the head of a salamander embryo is grafted onto its flank, extra limbs appear. (Modified from Balinsky, B.I. (1933). *Das Extremitätenseitenfeld, seine Ausdehnung und Beschaffenheit. Roux's Arch. EntwMech. Org.* **130**, 704–36.) (B) During the development of vertebrate embryos, the initiation of limb development requires fibroblast growth factor (FGF), whose activity leads to the expression of Sonic hedgehog, a protein that plays a key role in limb development. (C) Placing a bead impregnated with FGF on the flank of a chick embryo gives rise to an extra limb, just as transplanting the anterior head region to this position does in the case of the salamander.

order to answer the important questions. Such descriptions are measurements of the outcome of developmental processes but shed little light on the molecular elements involved or the nature of the processes they mediate. We do not think that one should dispense with such descriptions altogether. However, they only provide a framework for thinking about molecular interactions and, at best, a rudimentary language in which more mechanistic questions can be phrased.

Before embarking on an account of the molecular principles of development, it may be instructive to look briefly at the way in which the problem of embryonic development has been viewed and dealt with over the centuries. This will allow us to place the new 'molecular era' in a historical context and to introduce some classical embryological features and concepts. Although some of these classical terms are now being reinterpreted in the light of

molecular information, they still form part of the language of the subject and will be referred to at many points during the book.

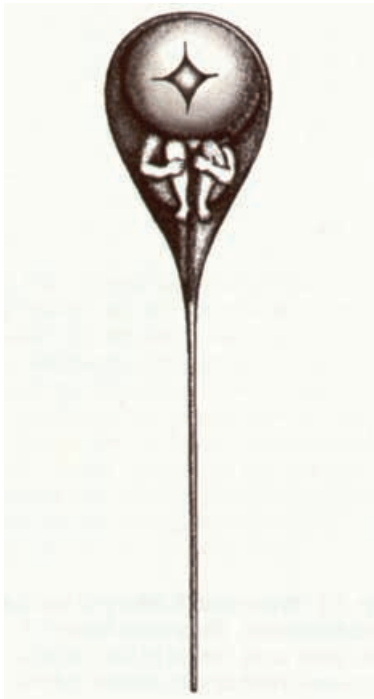
## Beginnings

The fundamental questions of developmental biology have always fascinated human beings. How can a complex animal emerge from a simple egg? And how are the characteristics that define individuals and species transmitted from one generation to the next? In the fourth century BC, Aristotle dissected chickens' eggs at different stages after fertilization, and in describing the development of a fully formed animal from a minute speck, he recognized what later became known as the principle of 'epigenesis'—that the development of an organism is a process of growth in size combined with increasing complexity and organization. With no means of observing the microscopic sperm and ovum, however, his view of the parental contributions to the offspring was that the embryo was formed by the

shaping influence of semen acting on the blood of the mother.

It was not until the development of microscopy during the seventeenth century that it became possible to explore developmental processes at a more detailed level. By the end of that century sperm had been seen by Antonie van Leeuwenhoek. William Harvey had asserted that the maternal contribution to the fetus was an egg rather than simply blood, and the anatomist Stensen had identified the ovarian follicles as the source of mammalian ova. But the ability to see more seems also to have unleashed the full powers of the human imagination, and despite Harvey's arguments to the contrary, the notion of epigenesis was eclipsed by the concept of preformation: the idea that either the sperm or the ovum contained a fully preformed organism, and that development consisted of no more than an increase in size of this 'homunculus' in the environment of the mother's uterus (Fig. 1.6).

As the quality of microscopes improved, the claims of the preformationists became less and less tenable, and Aristotle's principle of epigenesis reasserted itself: the homunculus did not exist; instead the embryo was sketched, outlined, coloured, and fleshed out little by little. The concept of epigenesis finally gained ascendancy in the



**Fig. 1.6. Preformationism.** The belief developed in the seventeenth century that the sperm contained a miniature replica of a human being, the homunculus, as shown in this drawing after Nicholas Hartsoeker (1694).



**Fig. 1.7. Epigenesis.** During the eighteenth century, the idea began to emerge that shape and form arise progressively during development. One of the first works to develop this concept was the 'Theoria Generationis' by Kaspar Friedrich Wolff (1759), which described different stages of chick development and the progressive emergence of form.



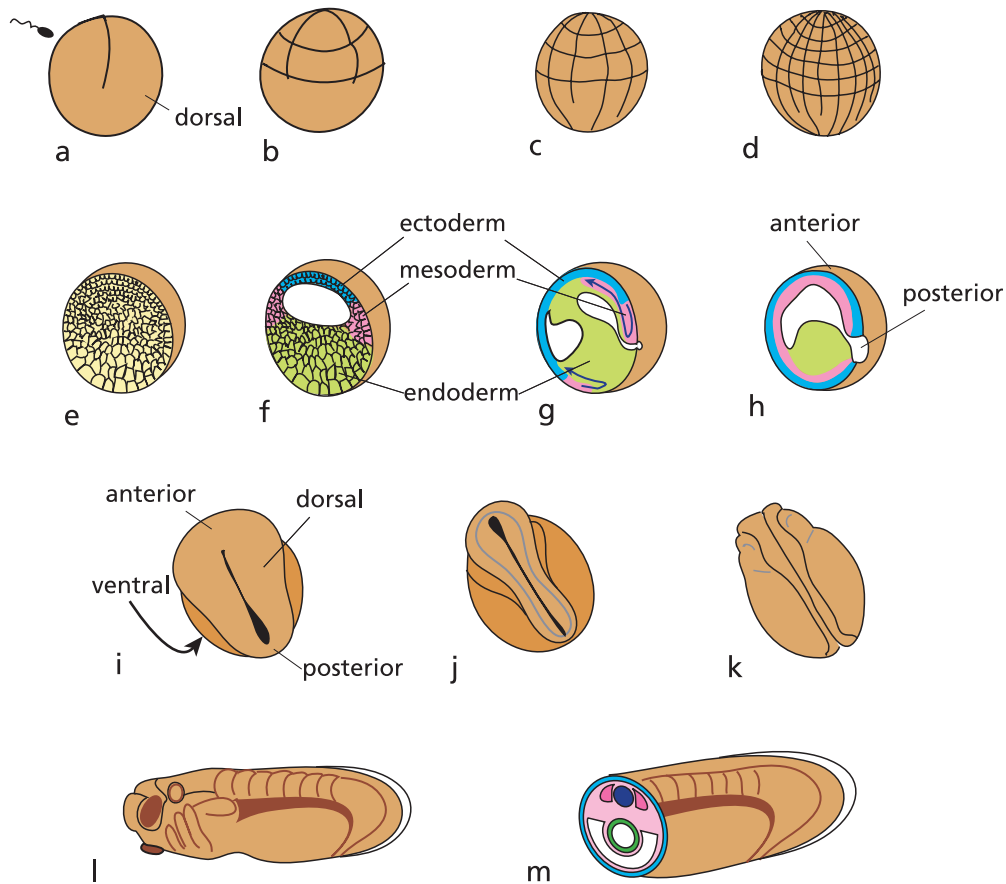
closing years of the eighteenth century, with the recognition of Kaspar Friedrich Wolff's minutely observed treatise on chick development (Fig. 1.7). Embryonic development consisted of a progressive and carefully orchestrated increase in morphological complexity, synchronized with growth in size.

## The heyday of descriptive embryology

By the nineteenth century, the discipline of embryology was firmly established, and embryologists were engaged in a ferment of observation and description, producing detailed accounts of the development of all manner of creatures from marine invertebrates to large, land-dwelling mammals. This century-long exercise brought with it its own set of principles, concepts, and terms, which became firmly embedded in the literature and in many cases have survived into the modern era. An important observation of this period was that, at the earliest stages of development, all animals look remarkably similar (Fig. 1.8).

**Fig. 1.8. Comparative embryology.** Composite from *The evolution of man* by Ernst Haeckel (4th edn, 1903) showing the development of (from left to right) a snake, a tortoise, a chicken, a dolphin, and a monkey. In these drawings the author emphasized the similarity of all embryos during the early stages of development, something that had been documented by Karl von Baer at the beginning of the century. However, whereas Haeckel mistakenly assumed that more complex organisms recapitulate the development of the ancestral ones, von Baer correctly interpreted the progressive similarities as departures from shared stages.

Furthermore, these very early stages appeared to follow a basic, stereotyped pattern, in which the fertilized egg first divides many times to generate a mass of cells that become a hollow ball, the blastula or blastoderm (Fig. 1.9). The blastula then undergoes a series of well-defined movements and invaginations—gastrulation—that folds the ball of cells within itself and converts what was essentially one layer into three. The resulting embryonic stage, the gastrula, consists of three groups of cells, also known as germ layers, each with a characteristic position relative to the others. The term 'germ layer' refers to the observation



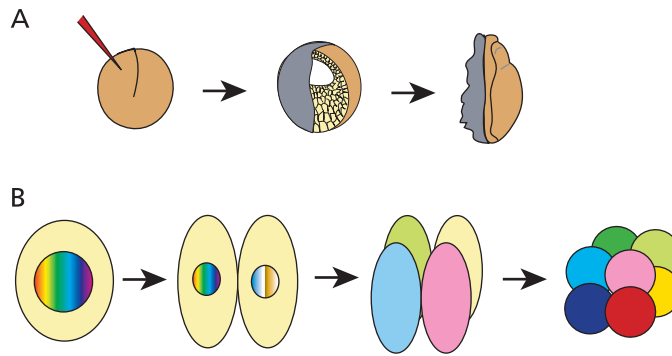
that each of these groups of cells acts as a rudiment, or 'germ', for a specific set of elements of the different tissues of the embryo (Fig. 1.9). The external layer was named the 'ectoderm', the internal layer the 'endoderm' and the intermediate layer the 'mesoderm'.

In most organisms the process of gastrulation reveals a clear outline of the basic body plan, with defined anterior (head), posterior (tail), dorsal (back), and ventral (belly) regions. From the gastrula stage onwards, these rudimentary tissues are expanded, shaped, and decorated in a huge variety of ways to create all the rich diversity of the animal kingdom. The classical nomenclature of these early developmental stages is still used today, and serves as useful way of referring to specific times and events in development.

During the second half of the nineteenth century the vogue for comparative embryology, together with the publication of Charles Darwin's theory of evolution by natural selection, brought questions of taxonomy and phylogeny to the fore. Developmental features, such as seg-

**Fig. 1.9. Different stages of development of the frog *Xenopus laevis*.** After fertilization the embryo cleaves several times (a–d) to generate a mass of cells (shown in transverse section in e–h). These cleavages are asymmetric because one side of the embryo (the vegetal side) is full of yolk, which impedes mitosis so that fewer and bigger cells are produced. After cleavage, concerted movement of the cells creates a cavity (f). Further movements of defined cell populations ensue (g) which, through the process of gastrulation, generate three layers of cells: the endoderm (green), the mesoderm (pink), and the ectoderm (blue), derived from different regions of the embryo. Gastrulation endows the embryo with a clear antero-posterior polarity (h, i). Further cell proliferation and a series of morphogenetic movements (i–k) give rise to the tadpole (l). A section through this stage of development (m) highlights the derivatives of the different germ layers.

mentation, body plans, and the organization of the germ layers, became part of the criteria for describing and classifying groups of organisms and proposing evolutionary pathways.



By this time, too, the description of developmental processes had reached the cellular level, and two things had become clear. The first was that one cell leads to many cells, in other words that cell proliferation is an important ‘engine’ of the developing system. The second was that proliferation was accompanied by a continuous but regulated increase in cell diversity. These observations prompted questions, such as ‘What instructions or mechanisms make one cell give rise to several different cells?’ and ‘How, during development, is all the information preserved to be passed on to the next generation?’

In the last two decades of the nineteenth century, August Weismann tackled both questions with a single proposal (Fig. 1.10). With remarkable prescience, he attributed a central role to the cell nucleus in both development and inheritance. He proposed that, as cells proliferate during development, different nuclear instructions or ‘determinants’ were allocated to different cells, causing them to form different tissues and organs, but that a group of ‘germ line’ cells, segregated from other cells, retained the full complement of nuclear determinants. From very early in development, the cells of the germ line were set apart so that they could not lose their capacity. This concept became known as the soma/germ line theory. Weismann’s insight was remarkable at many levels, as he also envisaged interactions between the cytoplasm and the nucleus in this process.

## Experimental embryology is born

Weismann’s proposals signalled a move away from a purely descriptive approach to embryology. His idea of determinants progressively allocated to different parts of the embryo was clearly testable and, in setting out to test it,

**Fig. 1.10. Experiment of Wilhelm Roux and its derived conclusions.** (A) Roux (1888) killed one of the cells at the two-cell stage of a frog embryo, and observed the development of the remaining cell. His interpretation of what he saw was that this cell developed into a half-embryo. This led him to conclude that during normal development, each of the two cells receives a certain amount of information about its developmental fate, and that the acquisition of this information means the loss of other information. (B) These observations fitted well with ideas of the time. According to this view, during development determinants for specific regions of the embryo were allocated as the cells divided. These determinants allocated specific developmental characteristics to the cells that received them.

Wilhelm Roux initiated a new, empirical approach that he called ‘developmental mechanics’. Taking a frog embryo at the two-blastomere stage, he killed one of the blastomeres and observed the development of the remaining one: it developed into something he interpreted as a half-embryo, thus apparently confirming Weismann’s theory (Fig. 1.10).

Among those excited by the possibilities offered by the new science of experimental embryology was the young biologist Hans Driesch. Driesch was intrigued by Roux’s experiments: could half-animals really exist? He decided to repeat Roux’s experiment but with a different organism, the sea urchin, and under different conditions. Unlike Roux, instead of killing one blastomere he merely separated the two and left them to develop in different dishes (Fig. 1.11). In his subsequent paper, he described his thoughts as he waited for the results of the experiment: ‘I awaited in excitement the picture that was to present itself in my dishes the next day. I must confess that the idea of a free-swimming hemisphere or a half gastrula . . . seemed rather extraordinary. I thought the formation would probably die. Instead, the next morning I found in

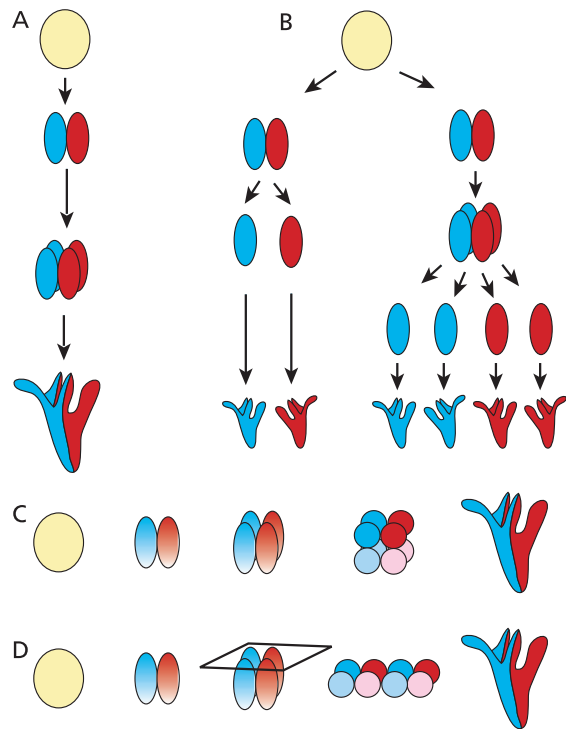
their respective dishes typical, actively swimming blastula of half size.'

This was a seminal moment in the history of developmental biology. Driesch had observed that early in development the blastomeres are 'aware' of whether they are part of a whole, or are the whole itself, and will tailor their development accordingly: if they are part of the embryo they will contribute to the whole, but if they are all that is available they will adjust, regulate, and give rise to the whole embryo (Fig. 1.11). This regulative property of the sea urchin embryo was later observed in many other embryos and in some cases was not restricted to the first division of the zygote. One possible explanation for the observations of Roux might be that he left some damaged tissue behind which affected the outcome of the experiment.

The result of Driesch's experiment poses an essential question about development, and one to which we still do not have a satisfactory answer: how does a cell 'know' when it is the whole or only part of the whole? The different behaviour of the blastomeres, depending on whether they are isolated or part of an embryo, suggests that during embryogenesis there are exchanges of information between cells that affect their developmental potential. This was underlined in a second experiment by Driesch (Fig. 1.11) which reinforced the view that the components of the embryo have a regulative ability. In this experiment, a zygote was allowed to develop until the eight-cell stage and then gently perturbed in such a way as to change the relative positions of the cells. This embryo developed completely normally, an outcome that would not have been possible had the fate of each cell been 'hard-wired' from the outset as suggested by the ideas of Roux.

These experiments introduced the concept, which has since been shown to be universal, that a cell within an embryo has both a 'prospective fate' (what it will become if left undisturbed) and a 'prospective potency' (its range of possible fates if the circumstances change). In addition they show that the potency of a cell is always wider than its fate, and make it clear that an understanding of how the potency of a cell arises and is regulated lies at the heart of developmental biology.

Driesch considered the possibility that during development cells acted as machines, and he wrestled to find a mechanistic explanation for what he had observed. He developed interesting and quite modern insights into the processes he was studying, for example, he wrote that 'development starts with a few ordered manifoldnesses, but the new manifoldnesses create by interactions new ones and these are able . . . to provoke new differences and so



**Fig. 1.11. Experiments of Hans Driesch on the sea urchin embryo.** (A) The development of the larval stage of the sea urchin relies on stereotyped events early in development which allow the fates of each of the blastomeres to be traced. (B) In 1892, Driesch reported his experiments on sea urchin embryos. He took embryos at the two- (*left*) and four-cell (*right*) stages, separated the cells and allowed them to develop as individual embryos. The result was always the generation of whole larvae; that is, cells that would have given rise to only one-half of the larva were, in isolation, capable of generating the whole of the larva. (C) The third cleavage of the sea urchin embryo is perpendicular to the plane of the first two and generates two tiers of four cells each. (D) By applying gentle pressure with a glass slide, Driesch changed the orientation of the third cleavage, thus altering the relative positions of the eight cells, then released the pressure and left the embryo to develop. The result was a normal larva.

on. With each effect immediately a new cause is provided and the possibility of a new specific action'. However, he failed to see a way towards a mechanistic explanation of development, largely because to find such an explanation he needed to know about molecules and genes and this knowledge was still many years in the future. In the absence of more concrete options he turned to abstract concepts and summed up the essence of what he had observed by describing the developing embryo as a

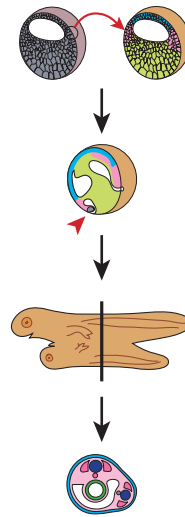
‘harmonious equipotential system’, that is, one in which all elements had the potential to develop into the whole but this potential was restrained by the need of the system to be ‘harmonious’.

## Morgan’s ‘deviation’: Genetics

As a consequence of the work of Roux and Driesch, the early twentieth century saw a steady stream of experimental manipulations that revealed the amazing potential of developing embryos for growth and regeneration. Hans Spemann has been quoted as claiming to have spent his life placing embryos in ‘ever more embarrassing positions’—to which one may add that the embryo was always one step ahead. As the list of embryological manipulations and their consequences started to rival the catalogue of descriptions of embryonic development, the important questions started to become clear. Most importantly, what was the material or molecular basis of the machine that runs development? Answers were harder to come by.

The issues that defeated Driesch remained. If embryos were to be thought of as machines, where was the engine? Where were the screws, the nuts and bolts? What was the fuel; what the laws that regulated the transformation of matter into energy? As no elements had been identified that would enable developing systems to be described in mechanical terms, concepts were developed to explain the behaviour and properties of their only obvious physical components: cells. Fields and organizers are two such concepts that arose during the early days of experimental embryology.

The ‘field’, a concept borrowed from physics, refers to a self-organizing group of cells and became popular as a concept in development at the beginning of the twentieth century. As in physics, a field is defined by the potentials of its constituent particles, in this case, cells, and can be used to explain the regulative properties of many embryos, or groups of cells within embryos. Organizers, discovered and named by Hilde Mangold and Hans Spemann, are special groups of cells that have the ability to determine the fates and organization of cells around them. The original ‘organizer’ was a group of cells in the frog embryo that directs the fates and arrangements of adjacent cells as they invaginate during gastrulation (Fig. 1.12). But these concepts, although they are useful in a descriptive sense and have survived the passing of time remarkably well, brought a mechanistic understanding no closer. If the organizer emits information, for example, what is the nature of this information and how is it processed?



**Fig. 1.12. Experimental demonstration of the activity of the organizer.** In a frog embryo, transplanting the leading edge of the mesoderm during gastrulation to a different position of an embryo induces a new focus of gastrulation (red arrow head) and, associated with it, a secondary axis. Other regions of the embryo do not show this activity, indicating that the cells of the organizer have special properties.

For some time it had been suspected that the nucleus contained a key to the problem; Weismann had said as much and the work of Theodor Boveri and Edmund Beecher Wilson, in particular, provided ample evidence in support of the idea that the nucleus contained the instructions for development. At this time, too, the role of the nucleus and specifically the chromosomes in the answer to another problem, that of heredity, was also becoming clear. In a career decision that had a vital impact on the history of modern developmental biology, Thomas Hunt Morgan, although originally an embryologist, abandoned experimental embryology and turned instead to the problem of how characters were transmitted from generation to generation: Morgan’s ‘deviation’, as the molecular geneticist Sydney Brenner has aptly called it, established the fundamentals of modern genetics, and the fruit fly *Drosophila melanogaster* as an invaluable experimental system. The concept of the gene as the unit of inheritance and the idea that genes were associated with chromosomes pre-dated Morgan’s work, but it was Morgan and his group who first used linkage to map the linear arrangement of genes on chromosomes, correlated the genetic maps obtained from

linkage studies with cytological maps of the chromosomes, recognized the effects of gene interactions, and systematically bred and maintained mutant stocks of *Drosophila*, thus laying the groundwork for the application of genetics to the study of basic cellular processes.

## The impasse

From the 1920s to the 1960s, biologists were engaged in unravelling the molecular basis of heredity and cell biology. From the point of view of heredity, a sustained focus on the nucleus paid off and, after the seminal experiments of Oswald Avery, Colin MacLeod, and MacLyn McCarty on DNA-mediated transfer of traits between bacteria, a long onslaught on the structure of DNA ended with the elucidation of the double helix by James Watson and Francis Crick. The ensuing discovery of the genetic code led to the idea that DNA makes RNA makes protein in a directional and largely irreversible manner: the ‘central dogma’ of molecular biology. The implication for genetics was that the information to generate the components of a cell and an organism, which had long been known to reside in the nucleus, were shown to lie in the DNA.

In parallel with this progress at the molecular front, there were several attempts to link genetics and development. Some thought that if this could be done there would be an answer to the questions posed by Roux and Driesch. But the problem was that despite the efforts of geneticists in describing developmental mutations there was at the time no simple way of bridging the gap between the mutation and the nature and normal function of the gene associated with it. For this there needed to be a way of pinpointing the gene, decoding the information it contained, and linking specific mutations with specific functional defects.

## Genetics meets molecular biology

Despite this impasse, the middle decades of the twentieth century did see an important advance in the use of genetics to study developmental processes, with work on the genetics of bacteria and their viruses, the bacteriophages. These studies sought to understand how bacteriophage lambda ‘chose’ between two alternative programs: integrating silently in a bacterial genome, or replicating its genome and destroying the bacterium. As a result of this work it became clear that not only phenotypic traits but

also strategic developmental decisions could be traced back to regulatory interactions controlling the expression of genes. François Jacob and Jacques Monod drew on this idea in their work on the regulation of bacterial adaptation to lactose utilization. They developed the model of the *lac* operon for the control of gene expression, a model that still permeates much of our thinking on the subject. In this model, a pattern of gene expression is generated through positive and negative regulatory proteins whose balanced interactions determine the activity of the genes.

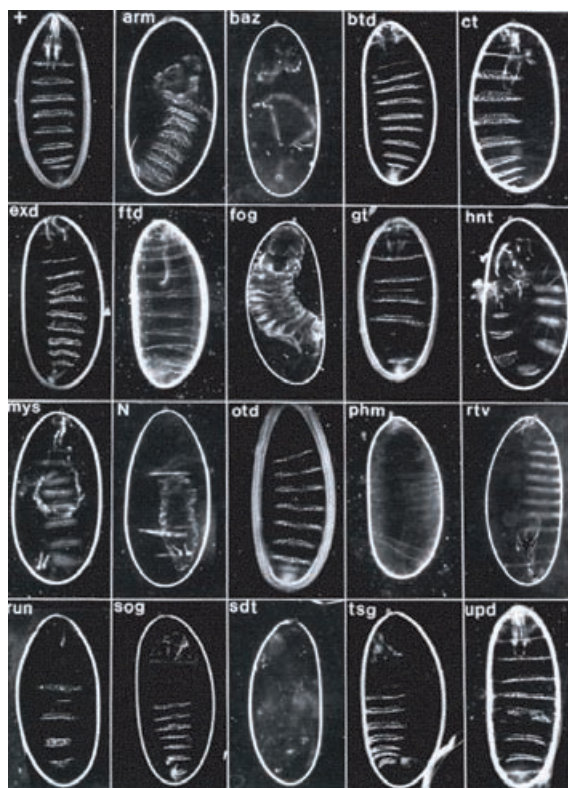
With these studies, and the subsequent identification during the 1970s of the genes and proteins involved, Genetics had met Molecular Biology. From the collecting of mutants, models began to emerge which portrayed development in terms of genetic circuits. In an approach pioneered by Ed Lewis, Eric Wieschaus, Christiane Nüsslein-Volhard and Gerd Jürgens on the fruit fly *Drosophila melanogaster*, the haphazard accumulation of developmental mutants was replaced by systematic screens to pick up mutants in specific processes (Fig. 1.13). From these, genes were identified that are involved not only in the generation but also the processing of information: the ‘generation’ because many of the genes encode proteins that are involved in defining cell- or tissue-specific characteristics, the ‘processing’ because others encode proteins involved in regulating, in time and space, the expression of those genes or the activity of the proteins they encode.

The nematode *Caenorhabditis elegans* joined *Drosophila* as a model organism with well-studied genetics and ease of experimental manipulation. With the development of molecular cloning it became obvious that the way forward was to clone, sequence, and analyse genes—an analysis that would yield not only the nature of the proteins but also of the developmental program itself. The culmination of this analysis has been the ability to sequence and analyse whole genomes (Fig. 1.14).

The reward for all this work has been the identification of the elements that form the basis of Driesch’s ‘harmonious equipotential system’, and the ability to see how they assemble into functional modules that create diversity and order, shape and function.

## Lessons from history?

This brief historical introduction shows how the central theme at the heart of developmental biology—the spatially and temporally regulated increase in cell number and diversity—has been perceived in different eras and in the light of different levels of knowledge. Broadly, it is possible



**Fig. 1.13. Patterning mutants of *Drosophila melanogaster*.**

Gallery of mutants obtained in a saturation screen for zygotic mutations affecting the pattern of the larval cuticle in *Drosophila*. The wild type pattern is shown at the top left corner (+) and each picture represents a mutation found on the X chromosome. (From Wieschaus, E., Nüsslein-Volhard, C., and Jürgens, G. (1984) Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*: III. Zygotic loci on the X chromosome. *W. Roux Arch. Dev. Biol.* **193**, 296–307.)

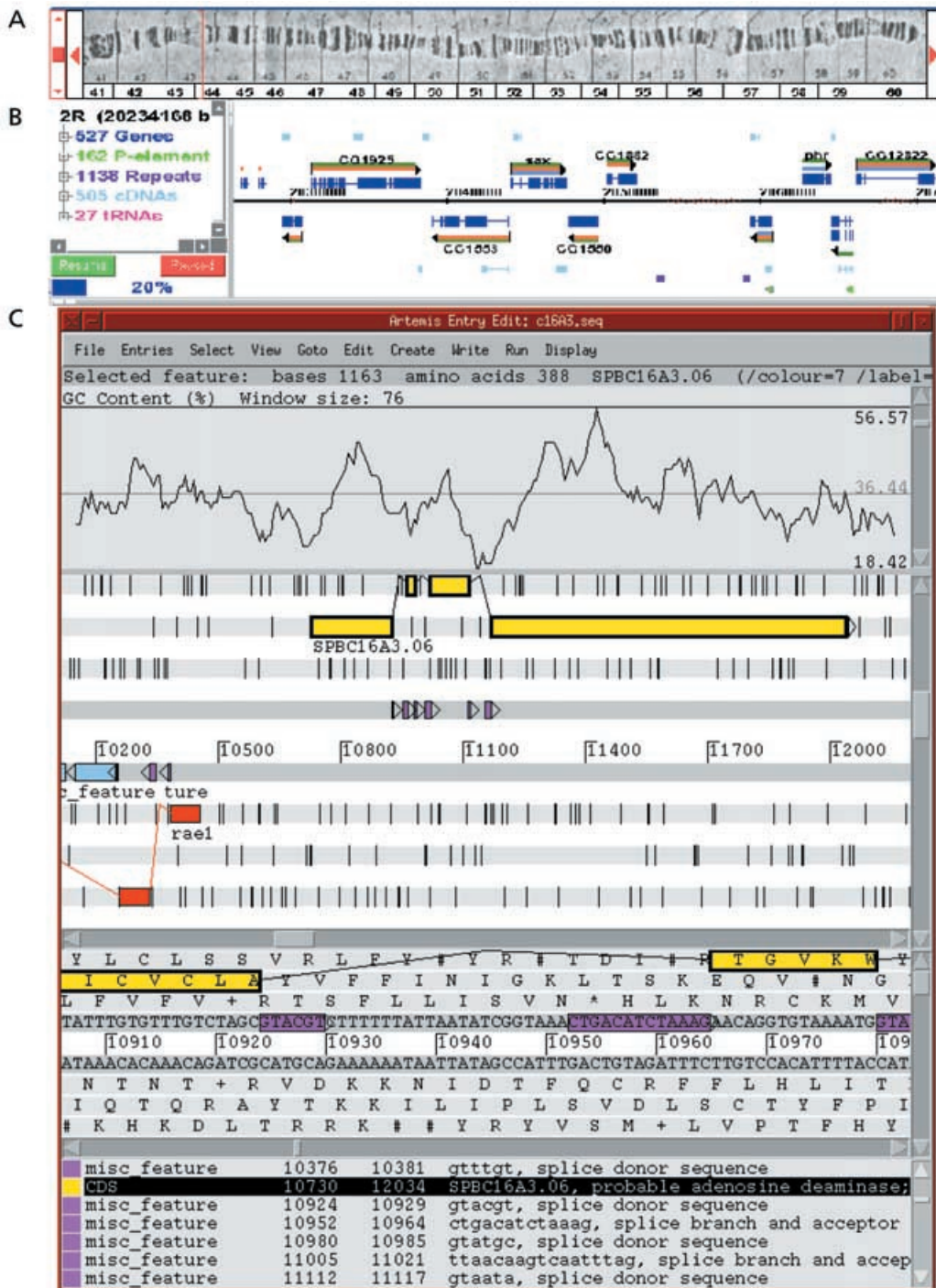
to discern three periods and one interlude. The first period was defined by the awareness that development is about change, not simply growth, while the second period was a call to arms to explain this change in mechanistic terms. These two phases were followed by an interlude or impasse while tools were developed to make this mechanistic approach possible. In the most recent period, a flood of information about the molecules that function during development has begun to make a detailed mechanistic understanding a reality.

At the end of each ‘phase’ there has been a brick wall because the progress made has led to questions whose answers required conceptual and technical advances. There are some interesting parallels here with the development of the physical sciences. Physics always demands a mechanical explanation for every observation but sometimes such explanations are not forthcoming in the way one would hope. For example, thermodynamics provides a conceptual understanding of certain processes like heat, but it is phenomenological: it invents quantities, such as enthalpy and entropy, that allow properties of the system to be measured, and that are needed to analyse macroscopic observations such as the formation of ice or the evaporation of water. However, thermodynamics itself does not provide a mechanistic explanation for these transitions of physical state. These explanations lie in the realm of statistical mechanics, which emerged long after thermodynamics was established. Statistical mechanics provides an account of the mechanisms that underlie the more conceptual ideas of thermodynamics (Fig. 1.15). It does this by inventing a new language and new concepts which allow us to deal with large numbers of particles and describe properties that emerge from their interactions.

In modern developmental biology, classical concepts such as ‘fields’ and ‘organizers’ are a bit like thermodynamic concepts: they are useful at a descriptive level but they do not provide an explanation of how their measurable properties are generated from their molecular components. As our knowledge of these components increases, it is very likely that a mechanistic understanding will emerge of many of the concepts that we use to represent developmental events. Along the way, it will be important to bear in mind the different levels of organization that we outlined earlier in this chapter (Fig. 1.2). Here might lie an important difference from the physical sciences, where usually only two levels have to be bridged: a macroscopic level and a microscopic one. In biology there is a scale of levels, each with emergent properties that depend on interactions with both the level above and the level below.

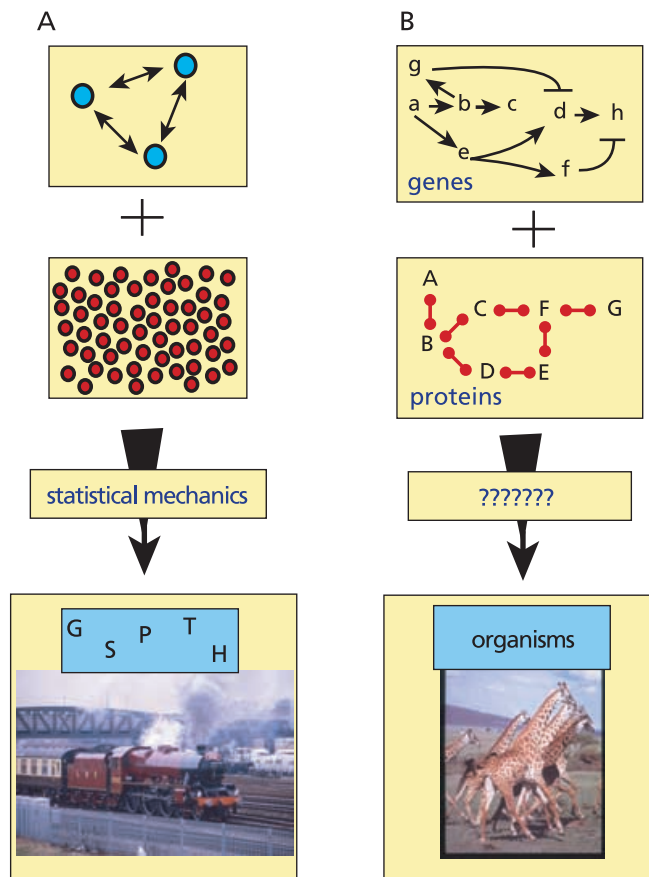
## About this book

A change is underway in how developmental biologists view their subject, in the sorts of questions they ask and in how they address these questions experimentally. This book stems from an appreciation of this change of perspective and attempts an approach to developmental



**Fig. 1.14. Genome analysis.** (A) Graphics from GadFly, the database for the *Drosophila* genome, displaying the right arm of the second chromosome (portrayed as a polytene chromosome). (B) Annotation of the predicted transcripts and genes that can be found in the region highlighted by a red bar on the polytene chromosome

(in subdivision 44). (C) A tool used to analyse genomic information is applied to one of the regions of DNA in subdivision 44. The programs within this tool find open reading frames and putative transcripts within large stretches of DNA and are used to create the maps illustrated in (B). (Image courtesy of S. Russell.)



biology that focuses, as far as possible, on the molecules and mechanisms that make organisms, rather than the more traditional emphasis on organisms and developmental systems.

The book is structured around the concept, introduced in this chapter (Fig. 1.2), that development involves increasing levels of complexity and organization and that in order to understand developmental processes we have to understand the organization of those levels. In Chapters 2–5, we describe the basic molecular elements of developing systems: the proteins and the DNA and how they create an information-processing unit within the cell. We begin by introducing the concept that the DNA contains a program that determines sequences of events through the proteins it encodes (Chapter 2). We then discuss how this information is decoded (Chapter 3) and move on to discuss the ways in which this decoding is regulated through cell interactions. We do this by introducing signals and receptors (Chapter 4) and seeing how these assemble into

**Fig. 1.15. A need for an integrated analysis of molecular events during development.** (A) In physics, thermodynamics uses variables, such as Gibbs free energy (G), enthalpy (H) or entropy (S), to describe temperature (T) or pressure (P). All these variables result from interactions between the particles that make up matter but the laws of ‘classical mechanics’ cannot be used to infer how interactions and collisions between particles generate S, H, T or P. The reason is that the calculations become too cumbersome, and meaningless in terms of what is being explained. This problem is circumvented by ‘statistical mechanics’ which provides an analytical tool and a language for applying the laws of mechanics to large numbers of molecules. (B) Developmental biology requires a related tool that will allow the properties of cells and tissues, as represented in the shape of organisms, to be described in terms of the interactions between genes and proteins. It is unclear, at the moment, whether the description of every interaction between genes or between proteins will provide such a picture. It is hoped that modern biology will find a conceptual analogue of statistical mechanics, which will allow us to understand developmental processes in terms of ‘average’ interactions between genes and proteins.

biology that focuses, as far as possible, on the molecules and mechanisms that make organisms, rather than the more traditional emphasis on organisms and developmental systems. The book is structured around the concept, introduced in this chapter (Fig. 1.2), that development involves increasing levels of complexity and organization and that in order to understand developmental processes we have to understand the organization of those levels. In Chapters 2–5, we describe the basic molecular elements of developing systems: the proteins and the DNA and how they create an information-processing unit within the cell. We begin by introducing the concept that the DNA contains a program that determines sequences of events through the proteins it encodes (Chapter 2). We then discuss how this information is decoded (Chapter 3) and move on to discuss the ways in which this decoding is regulated through cell interactions. We do this by introducing signals and receptors (Chapter 4) and seeing how these assemble into

networks that can process molecular information by creating relationships between the membrane and the nucleus that allow cells to receive inputs and produce outputs, to integrate and to calculate (Chapter 5).

In Chapters 6–9, we explore how the molecular networks act within cells to create ‘routines’ that can be used to perform developmental operations. First (Chapter 6), we introduce basic cellular activities, such as adhesion, movement, and cell interactions, that modulate much of the behaviour of cells in developing embryos. In Chapter 7, we continue this trend by discussing cell division and cell death and the way they contribute to development. In Chapter 8, we see how large-scale regulated cell division begins to create cell diversity and, in Chapter 9, how long- and short-range cell interactions contribute to this diversity.

With these first two parts of the book we hope to lay down the molecular and cellular elements that operate in developing embryos. In the last part of the book we explore these basic mechanisms in action. In Chapter 10, we study what is basically a problem of change over the single dimension of time: how are specific cell types generated during development? In the following two chapters we gradually add further, spatial, dimensions, to see how different cell types become organized in what are essentially a series of two- (Chapter 11) or three- (Chapter 12) dimensional patterns.

Unlike most traditional developmental biology texts, the book does not contain a separate chapter or section devoted to discussing the emergence of body plans, that is the laying out early in embryogenesis of the coordinates that act as a reference system for the development of particular embryos. The reason is that we do not see this process as a special one that is different from the basic organization of

a limb or the patterning of epithelial cells. We see body plans rather as examples of the outcomes of molecular and cellular interactions and, as such, aspects of the ways in which they can be set up will figure in several places throughout our discussion.

One obvious omission from this book is plant development. This is not because we think plants develop in a way that is fundamentally different or that uses completely different elements. On the contrary, the findings of the last few years have shown that plants follow essentially the same developmental principles and they have made their own contributions to the general concepts that we have discussed. Plants also have their own peculiarities, however (as do animals, from the point of view of plants). Perhaps unjustly, we follow a fairly widespread bias and base our discussion on examples from animal development. We nevertheless hope that this book will be of general use to all developmental biologists.

Although the focus of this book is on animal development, several of the examples we discuss in detail, particularly in the first half of the book, come from the yeast *Saccharomyces cerevisiae*. The reason for this is that, largely because of its small genome and its amenability to genetic approaches, the detailed molecular analysis of biological processes is in many cases more advanced in yeast than in higher eukaryotes. Yeast does undergo some simple, regulated developmental events, such as mating type switching and sporulation, that are analogous to developmental events in higher eukaryotes such as animals. This, together with the remarkable evolutionary conservation of many of the individual elements involved in biological processes in all eukaryotes, suggests that lessons learned in yeast will be relevant in higher eukaryotes as well.

## SUMMARY

1. Biological systems are organized into a hierarchy of functional modules that are linked through their component elements.
2. The components of each module contain the information to generate the next higher level of complexity and to feed back to the lower one. For example, proteins, the fundamental functional components of cells, feed back information both to the genes that code for them and to other genes, creating functional interrelationships.
3. Development relies on the generation of large numbers of cells which are diversified in a regulated manner and organized in space.
4. The generation of complexity during development is a progressive process that relies on the organized unfolding of the information contained in each level of complexity. Because there is

constant feedback between the different levels, this process is an open one that carries on throughout the life of an organism.

5. The first major conceptual advance in the history of developmental biology was the realization that development is about change, not just growth. Attempts to explain development in mechanistic terms were, however, hampered by lack of the right experimental tools.
6. In the modern era, a flood of information about the molecules that function during development has begun to make a detailed mechanistic understanding of this process a reality.
7. Genetics contributes to this understanding by enabling us to interfere with the activity of proteins and their interactions and infer, from the results, how these processes normally function. Functional genomics adds to this the ability to analyse simultaneously the functions of whole batteries of genes. Molecular and cell biological techniques allow us to probe, *in situ*, the activity of proteins and their effects on the properties and behaviour of cells.
8. A major challenge for the future is to devise languages that can enable us effectively to describe and understand the dynamic molecular and cellular events that form the basis of development.

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