

Models and Methods for Studying Neural Development

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1.1 What is neural development?

Neural development is the process by which the nervous system grows from its first beginnings in the embryo to its completion as a mature functioning system. The mature nervous system contains two classes of specialized and closely interacting cells: **neurons** and **glia**. Neurons transmit signals to, from and within the brain: their axons transmit electrical signals and they communicate with other cells via **synapses**. There are many types of neuron with specialized shapes and functions, with cell bodies that vary in diameter from only a few micrometers to around 100 micrometers and with axons whose lengths vary from a few micrometers to more than 1 meter. There are also different types of glial cell. The interactions between neurons and glia are very precise and they allow the nervous system to function efficiently. Figure 1.1 shows a beautiful example of the complex structures created by interacting neurons and glia, in this case a microscopic view of a labelled node of Ranvier, which allows rapid signalling in the nervous system.

The great molecular, structural and functional diversity of neurons and glia is acquired in an organized way through processes that build on differences between the relatively small numbers of cells in the early embryo. As more and more cells are generated in a growing organism, new cells diversify in specific ways as a result of interactions with pre-existing cells, continually adding to the organism's complexity in a highly regulated manner. The development of an organism is a bit like the development of human civilization (allowing for the obvious difference that organismal development repeats over and over again). In both, population size and sophistication (be it humans on earth or cells in an organism) grow hand-in-hand, each stage adding further layers of complexity to previously generated structures, functions and interactions. The mechanisms that regulate cellular actions and interactions during development are often described using terms commonly applied to human activities. We shall highlight this at several places throughout the book where analogies might be helpful.

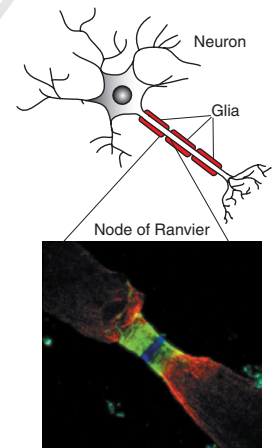


Figure 1.1 A node of Ranvier: these highly organized structures, formed as a result of interactions between axons and glia, are essential for speeding up the transmission of electrical signals along axons. In this single fibre from the mouse spinal cord, sodium channels (blue) are sandwiched between the regions where axons and glia form junctions (called axoglia junctions) (green), which are, in turn, flanked by potassium channels (red). This picture is courtesy of Peter Brophy and Anne Desmazieres, University of Edinburgh, UK.

To understand how organisms develop we need to know how cells in each part of the embryo develop in specific and reproducible ways as a result of their own internal mechanisms interacting with an expanding array of stimuli from outside the cell. Many laboratories around the world are researching this area. Why?

1.2 Why research neural development?

1.2.1 *The uncertainty of current understanding*

One reason for researching neural development is that we still know relatively little about it. In this book we shall try to explain some of the main events that occur during neural development and, in particular, the mechanisms by which those events are brought about, in so far as we understand them. It is important, however, to appreciate that much of what we present, particularly our understanding of molecular mechanisms, is best thought of as continually evolving hypotheses rather than established facts. The biologist Konrad Lorenz once stated that ‘truth in science can be defined as the working hypothesis best suited to open the way to the next better one’; this is highly appropriate in developmental neurobiology.

Some of our understanding is incomplete or may be shown by future experiments to be inaccurate. We have tried to highlight issues of particular uncertainty or controversy and to indicate the limits of our knowledge, since it is at least as important and interesting to acknowledge what we do not know as it is to learn what we do know. Much of the excitement of developmental neurobiology arises from the mystery that surrounds Nature’s remarkable ability to create efficiently and reproducibly neural structures of great power.

One reason that we still know relatively little about the mechanisms of neural development is the sheer size and complexity of the finished product in higher animals. During the development of the human brain, for example, about 100 billion cells are generated with about 1000 trillion connections between them; if this number of connections is hard to visualize then consider that it might roughly equal the number of grains of sand on a small beach. Although cells and connections with similar properties can be grouped together, there is still great variation in their molecular make-ups, morphologies and functions throughout the nervous system. In reading this book you will see that many of our hypotheses about neural development are formulated at the level of tissues or populations of cells rather than individual cells and their connections, particularly in higher mammals. Only in very simple organisms containing a few hundred neurons (e.g. in some worms) do we fully understand where each cell of the adult nervous system comes from and even then we do not know for sure what mechanisms determine how each cell and its connections develop. We still have a long way to go to gain a profound understanding of the molecular and cellular rules that govern the emergence of cells of the right types in the right numbers at the right places with the right connections between them functioning in the right ways.

1.2.2 Implications for human health

Just because we do not know much about a subject is not sufficient reason to want to invest time and resources in researching it further. However, there are many practical reasons for wanting to know more about the ways in which the nervous system develops. A better understanding should help us to tackle currently incurable diseases of the nervous system. Many congenital diseases affect neural development¹ but their causes are often unknown; some examples of such diseases will be given later in this and in subsequent chapters. Numerous relatively common psychiatric and neurological diseases, such as schizophrenia, intellectual disabilities, autisms and some forms of epilepsy (Figure 1.2), are now thought to have a developmental origin, but the mechanisms are poorly understood. Knowledge of how cancers form should be helped by a better understanding of normal development; the uncontrolled growth of cancer cells is often attributed to abnormalities of the same molecules and mechanisms that control growth during normal development. Regarding the development of

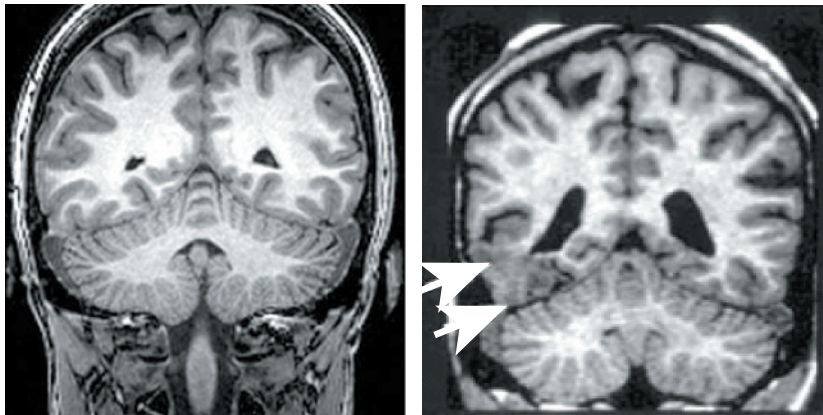


Figure 1.2 Schizophrenia, intellectual disabilities, autisms and epilepsies are neurological disorders affecting about 3–7% of people. Based on epidemiological and neurobiological evidence, schizophrenia is now believed to be a neurodevelopmental disorder with a large heritable component. Many possible susceptibility genes have been identified, but how abnormalities of these genes cause the symptoms of the disease is unknown. Similarly, autism spectrum disorders and intellectual disabilities are highly heritable and many of the known genetic causes seem to regulate the formation of synapses. Malformations of cerebral cortical development are among the commonest causes of epilepsy. Some are large defects that would be obvious to the naked eye whereas others would only be seen at a microscopic or molecular level. They are a consequence of a disruption of the normal steps of cortical formation, for example defective migration of neurons, and can be environmental or genetic in origin. A large number of malformations of cortical development have been described, each with characteristic pathological and clinical features. An example of a large congenital defect causing epilepsy is shown in the scan of a patient's brain on the right (between the arrows): for comparison, a scan of the brain of a normal person is shown on the left. This picture is courtesy of Professor John S. Duncan and the National Society for Epilepsy MRI Unit, UK.

¹ For a comprehensive compendium of human genes and genetic diseases, see <http://omim.org/> about. For an interesting review of neurodevelopmental disease and its impact, try Stoeckli, E.T. (2012) What does the developing brain tell us about neural diseases? *European Journal of Neuroscience*, 35, 1811–1817.

possible new treatments, it has been suggested that a diseased brain might be repaired by replacing dysfunctional genes or implanting new cells into the nervous system. Implanted cells would need to recapitulate a developmental programme allowing their survival and functional integration into the nervous system and its circuitry. How this might be achieved is currently unclear, but research on normal developmental mechanisms might help.

1.2.3 Implications for future technologies

Another, perhaps unexpected, motivation for understanding how the brain develops comes from the drive to revolutionize computer technology, to improve robotics and to generate autonomous machines able to make decisions. The application of current manufacturing methods to build much more complex computers than exist at present will need to overcome exponential increases in the production cost of ever smaller and faster circuits. In contrast, evolution has produced brains of enormous computing power that self-construct with great efficiency. Can lessons learned from studying the way the brain constructs itself be used to invent new, more efficient ways of generating computers by having them self-construct? Maybe this sounds like science fiction, but international organizations are taking it seriously enough to put large amounts of money into research aimed at establishing whether it might be possible.²

1.3 Major breakthroughs that have contributed to understanding developmental mechanisms

The twentieth century saw breakthroughs that have added greatly to our knowledge of how the nervous system develops. Most notable were the discovery of the structure of DNA and the development of methods for manipulating the functions of genes. We assume that the reader is familiar with the structure and function of DNA; methods for manipulating gene function will be outlined later in this chapter.

Another critically important advance in the twentieth century was the realization that, although animal species differ hugely in size and structure, the mechanisms by which their development is controlled are remarkably highly conserved. Many of the genes that control the development of relatively simple invertebrates have clear **homologues** in higher mammals, including primates. This means that by studying the mechanisms controlling the development of simple experimentally tractable organisms we can learn much of relevance to human development, which cannot be studied extensively for practical and ethical reasons.

A small handful of animal species, referred to as **model organisms**, are used in most developmental neurobiological research because each has

homologue a gene or structure that is similar in different species since it was derived from their common ancestor during evolution.

²See Douglas, R. (2011) Constructive cortical computation. *Procedia Computer Science*, 7, 18–19, which links to a recording of an interesting lecture on this topic.

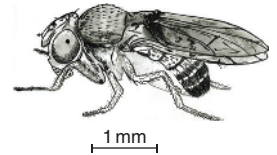
clear advantages for certain types of research. The following sections describe the best-studied of these and their advantages; there are many others that have been used less frequently.

1.4 Invertebrate model organisms

1.4.1 Fly

One of the most famous invertebrate model organisms for developmental genetics is the fruit fly, *Drosophila melanogaster* (right), a small insect often found around rotting fruit. *Drosophila* has a life cycle of only 2 weeks and is cheap and easy to breed in large numbers. The eggs can be collected easily and embryogenesis takes only 24 hours. Much of the research that has been done with this organism started when scientists established **lines** of mutant flies with abnormal **phenotypes** (Figure 1.3). The analysis of these mutant lines led to the discovery of the genes that were mutated in each case. By finding the genetic defects that caused the abnormal phenotypes, researchers gained knowledge of the functions of critical genes.

Working from phenotype to gene is often referred to as **forward genetics**. Box 1.1 illustrates in more detail how lines of *Drosophila* with abnormal phenotypes can be generated in a so-called forward genetic screen. *Drosophila* contain up to 17 000 genes, many of which are named, sometimes fancifully, after the phenotype that results from their mutation; in comparison, the human **genome** contains around 20 000–25 000 genes. Remarkably, about 50% of fly protein sequences have mammalian homologues. *Drosophila* is being used increasingly as a model organism in which



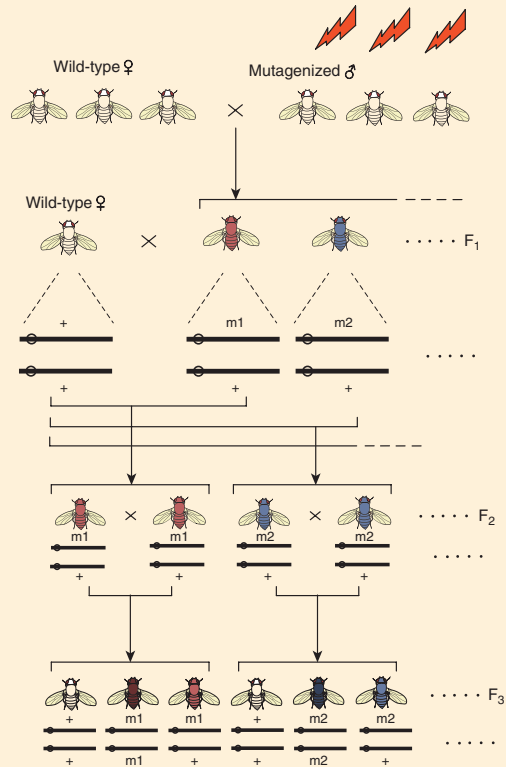
line a collection of organisms related by breeding that is relatively pure genetically because of continued inbreeding and artificial selection.

phenotype the observable characteristics of an organism, such as its physical appearance or behaviour.



Figure 1.3 Two fruit flies face each other: the fly on the right is a normal (wild-type) fly, the one on the left is a mutant. In the mutant, a gene that is essential for the formation of eyes is defective. Flies lacking this gene do not develop eyes. The gene in question, *Pax6*, can be found in virtually all animals: in humans, flies, molluscs and even very simple worms. The *Pax6* gene is also called *eyeless* in *Drosophila*, since *Drosophila* genes are often named after their mutant phenotype: thus, somewhat confusingly, the *eyeless* gene is *required* to make the eye. This striking image is reproduced here with permission and is the copyright of Jürgen Berger and Ralf Dahm, Max Planck Institute for Developmental Biology, Tübingen, Germany (www.ralf-dahm.com).

Box 1.1 Forward genetics: working from phenotype to gene



This diagram shows a strategy that has been widely used in *Drosophila* to mutate randomly a large number of genes and then screen for those mutations that produce abnormal phenotypes in the offspring. Once such a screen is done, the experimenter can go on to identify genes whose mutation causes abnormal phenotypes of interest. Mutations are usually induced by feeding male flies the potent mutagen ethyl methane sulphonate or by X-ray irradiation (top right). This induces mutations in the male germ cells. These mutagenized males (blue and red colouring indicates flies with different mutations) are crossed to normal wild-type females (top left; two chromosomes are shown, wild-type chromosome marked +). This generates an F₁ population containing a large number of flies, many of which will be **heterozygous** for a random mutation (m₁, m₂, ...). At this stage, the experimenter will only know of flies carrying **dominant** mutations that generate phenotypes in the heterozygotes. Each F₁ fly is crossed to wild-type females (second row) to generate populations of F₂ flies (third row). Sibling mating within these populations will generate populations of F₃ flies (final row), some of which will be **homozygous** for each mutation, allowing phenotypes due to **recessive** mutations to be identified. In this way, the experimenter can establish many lines of *Drosophila* carrying dominant or recessive mutations generating phenotypes of interest.³ Similar approaches can be used in other species. Amongst mammals, the mouse is the species of choice and many lines carrying naturally occurring mutations or mutations induced by chemicals or radiation have been established. Once phenotypes of interest have been identified by these screens, the process of identifying the genes whose mutation causes them begins. Descriptions of how this is done can be found elsewhere.⁴

heterozygous (for a particular genetic feature, e.g. a gene or mutation) describes the situation where the two copies of the feature in question are different.

homozygous (for a particular genetic feature, e.g. a gene or mutation) describes the situation where the two copies of the feature in question are the same.

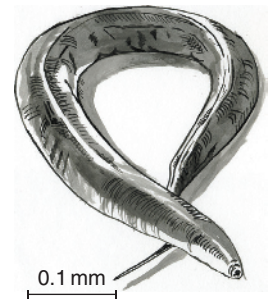
to study human disease:⁵ 75% of human disease-associated genes have fly homologues. The importance of research on this organism was recognized in 1995 by the award of a Nobel Prize to Ed Lewis, Christiane Nusslein-Volhard and Eric Wieschaus for their discoveries on the genetic control of early embryonic development.⁶

As well as being ideal for forward genetics, *Drosophila* can also be used for the opposite type of approach, called **reverse genetics**, in which one starts with an interesting-looking gene and manipulates its activity so as to learn about its function. It is possible to activate specific genes in *Drosophila* using a method called the GAL4/UAS system. Box 1.2 outlines how the GAL4/UAS system works.⁷ It allows specific genes to be activated in a spatially and temporally controlled manner and it can be used in a variety of ways. For example, genes normally found in the *Drosophila* genome can be activated by the experimenter to discover what they do (called a **gain-of-function** approach). Alternatively, the method can be used to activate genetic inhibitors manufactured by the experimenter to produce molecules that block the actions of a specific *Drosophila* gene (called a **loss-of-function** approach). How such blocking molecules work is discussed in more detail below (see Figure 1.4).

1.4.2 Worm

Another invertebrate model organism even simpler than *Drosophila* whose analysis has contributed greatly to understanding mechanisms of neural development is the nematode worm, *Caenorhabditis elegans* (*C. elegans*, right), which lives in the soil and feeds on bacteria and fungi. It is easy to maintain in the laboratory and viable organisms can be stored frozen. Its development is completed rapidly within 2–3 days, it is transparent and its anatomy is known in precise detail: for example, all of its neurons and the connections between them are known. Furthermore, its development is highly stereotypical and, from **zygote** to adult worm, we know all the cell divisions that occur to generate a particular differentiated cell (i.e. we know the full details of each cell's **lineage**). Detailed knowledge of cell lineage is unusual and valuable; in most model species indirect methods must be used to deduce lineages and knowledge is usually far from complete. Further discussion of cell lineage can be found in Box 1.3.

In *C. elegans*, for any cell at any point in normal development it is possible to know what that cell will do and what it will become, that is its **fate**. Against this background of precise morphological knowledge, it is relatively straightforward to study gene function by forward or reverse genetic methods, that is by generating mutant worm strains or by interfering with the actions of



zygote a fertilized cell that gives rise to an embryo.

³The reader should also be aware that this is a simplified description of only one type of screen and for a more comprehensive review we suggest St Johnston, D. (2002) The art and design of genetic screens: *Drosophila melanogaster*. *Nature Reviews Genetics*, 3, 176–188.

⁴For example, Kile, B.T. and Hilton, D.J. (2005) The art and design of genetic screens: mouse. *Nature Reviews Genetics*, 6, 557–567.

⁵Botas, J. (2007) *Drosophila* researchers focus on human disease. *Nat. Genet.*, 39, 589–591.

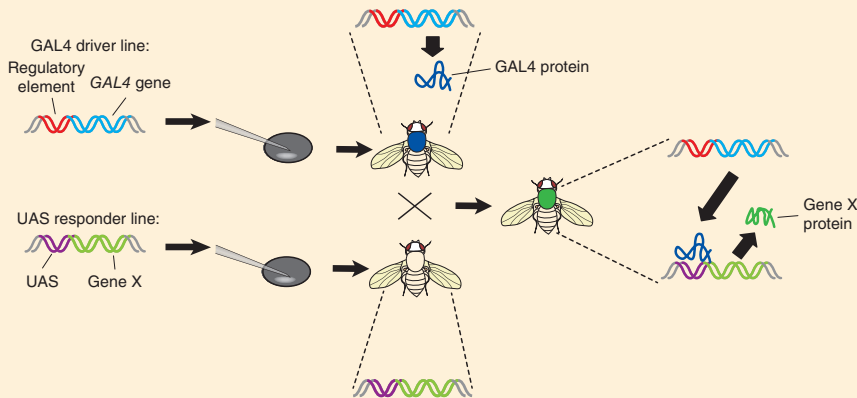
⁶www.nobel.se/medicine/laureates/1995/illpres/index.html [20 November 2010].

⁷See also Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, 118, 401–415.

Box 1.2 Reverse genetics: working from gene to phenotype



The GAL4/UAS system is used by many researchers to study the function of genes in *Drosophila* (it has also been used in other species such as frogs and fish). The system has two parts, each contained in a different line of organisms. The two parts are brought together by crossing the two lines, resulting in a line in which a specific gene is activated in a specific set of cells.



- (1) The first thing needed is a line of **transgenic** organisms, called the driver line, in which a protein called GAL4 is produced selectively in the cells where the experimenter eventually wants the gene of interest (X) to be activated. If such a line does not already exist (see below), the experimenter makes one by constructing a piece of DNA containing: (i) the *GAL4* gene, (ii) sequences that will activate the *GAL4* gene in the desired pattern (called regulatory elements) and (iii) a sequence called a P-element (not shown), which allows the whole piece of DNA to enter the genome when it is injected into an embryo. Making this piece of DNA requires the selection of a suitable regulatory element that will activate the *GAL4* gene in the desired pattern. This selection would be based on prior knowledge from research on the regulatory elements that normally activate specific genes in specific patterns. How genes are controlled by regulatory elements is described in Chapter 3. (Note that *GAL4* is a yeast gene engineered into *Drosophila*, where the gene is italicized and the protein is not; see section on Conventions and Commonly used Abbreviations at the start of this book.)
- (2) The second line (called the responder line) is made by generating a piece of DNA with three components: (i) the sequence of the gene to be activated (X), (ii) a sequence that will activate this gene *only* if it is bound by GAL4 (called the upstream activation sequence, or UAS) and (iii) a P-element (not shown) to carry the DNA into the genome. Gene X will not be activated in this line since GAL4 is a yeast protein that would not normally be there.

Once these two lines have been generated, they are crossed to achieve activation of the gene of interest (X) in the desired pattern. This might seem a long-winded way of doing things: for example, why not put the gene to be activated (X) directly under the control of sequences that will activate it in the desired pattern? There are several reasons for this; a main one is that large numbers of GAL4 driver lines have already been made and, in practice, the experimenter should only need to make the responder. Once the responder line is made it can then be crossed to a large variety of existing GAL4 driver lines, increasing the flexibility of the experiment.

transgenic describes an organism whose genetic material has been modified.

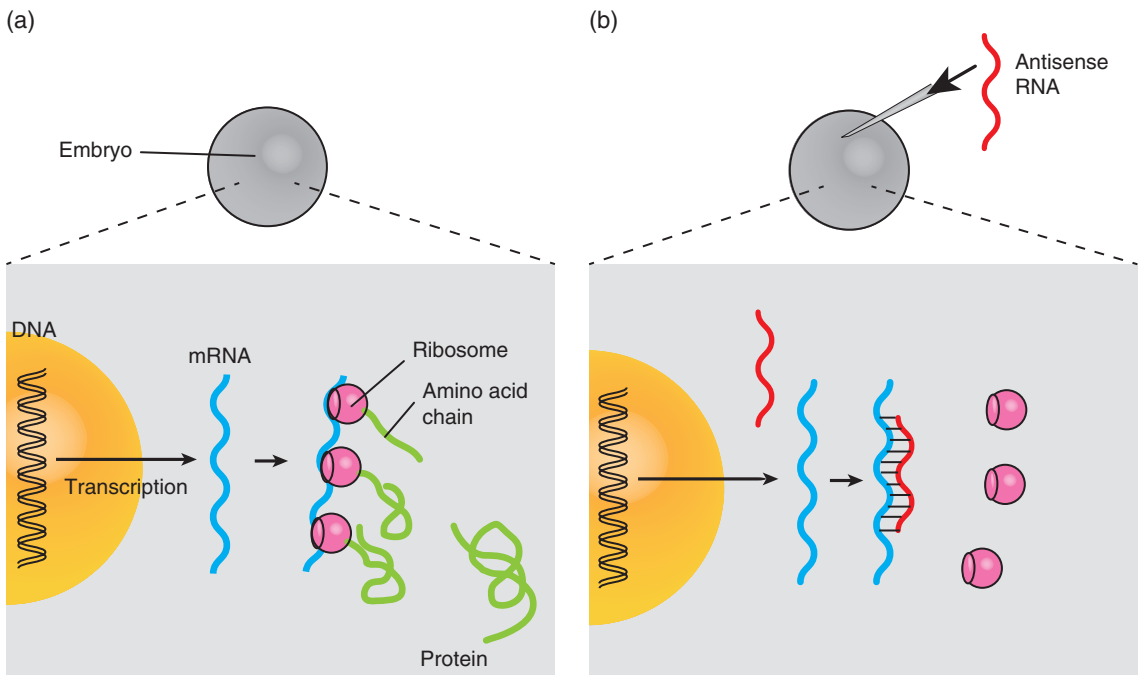


Figure 1.4 Reverse genetics RNA interference can be used to block gene function experimentally. (a) Inside normal cells, genes are transcribed to make single-stranded messenger RNA (mRNA) that is translated by ribosomes to generate specific proteins. (b) To block gene function, **antisense RNA** molecules with sequences complementary to the sense sequences of specific mRNAs are introduced into cells where they interact with their target mRNAs and block their translation. Many types of antisense molecule have been developed. They fall into two broad groups: after binding to target mRNA, some cause its enzymatic degradation whereas others can block its translation. For example, antisense molecules called morpholinos, which have been exploited very successfully in studies of *Xenopus* and zebrafish development, are examples of the latter. As well as being experimental tools, antisense molecules have therapeutic potential for treatment of human diseases. The development of antisense methods to regulate gene function experimentally or therapeutically was followed by the discovery of a wide range of small RNA molecules called microRNAs that are generated naturally by cells and act as physiological antisense molecules (see Section 3.8.4 in Chapter 3). In this diagram, the antisense RNA is introduced by microinjection, but there are many other ways such as **electroporation** (see Figure 1.7 later) or the use of viruses. Each method has pros and cons and which one is best depends on factors such as the numbers and types of cells to be targeted, the species and the age of the organism. In the future, the use of inhibitory RNA molecules described here is likely to be replaced increasingly by the use of RNA-based approaches that disrupt gene function by mutating the gene in the DNA (e.g. using the **CRISPR/Cas9** system; see Section 1.5.4).

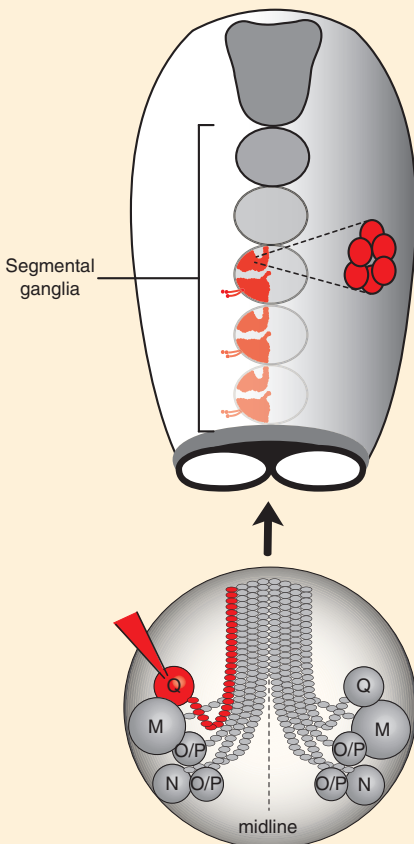
specific genes (for example, using RNA interference methods; see Figure 1.4). Since one of the sexes of *C. elegans* is **hermaphrodite** (the other is male), mutant worms that are severely defective and would be unable to mate can still be bred via self-fertilization. In 2002, Sydney Brenner, Robert Horvitz and John Sulston were awarded a Nobel Prize for work on the genetics of *C. elegans* development.⁸ Since many of the genes in *C. elegans* have functional counterparts in humans and whole biochemical pathways are often conserved, research on this relatively simple organism has given us a major insight into our own development (for example, in work on naturally occurring cell death described in Chapter 9, Section 9.3).

hermaphrodite an organism with both male and female sexual characteristics and organs.

⁸http://nobelprize.org/nobel_prizes/medicine/laureates/2002/horvitz-lecture.html [20 November 2010].

Box 1.3 Cell lineage

Cell lineage is a term used to describe the sequence of cell divisions that have given rise to any particular cell in an organism. To describe the lineage of a cell, therefore, we must observe directly, or infer by more indirect means, the divisions that have generated it. Direct observation is feasible in simple organisms. The first cell lineage studies were done by Charles Whitman in 1870 on leech embryos; since then, direct observations have been used to follow cell lineages in other invertebrate species such as *C. elegans* and *Drosophila*. In some situations in the analysis of invertebrate lineages, and in most situations in the analysis of vertebrate lineages, it is not possible to observe lineages directly. In such cases, the use of molecular markers carried through the generations from a cell to its descendants can help define cell lineages; suitable markers include dyes or reporter molecules (for example green fluorescent protein, see Box 1.4 and Chapter 7, Section 7.2) whose genes are incorporated into the genome of selected cells. The latter have the advantage that they are not diluted with each round of division. In simple organisms such as the leech (see below) and *C. elegans*, patterns of cell division are very similar or identical from individual to individual, and the lineages of the cells that are generated in this way are described as invariant. In the complex nervous systems of higher organisms it is hard to know the extent to which lineages are invariant. It is likely that lineages in higher organisms show greater variation because, as we shall see in later chapters, the fates of their cells rely heavily on signalling between cells and this process is inherently susceptible to variation from individual to individual.



At the bottom is shown the early leech embryo developing from bilateral sets of teloblasts, five on each side named M, N, O/P, O/P and Q. Dye injection (red) into a teloblast labels the cells generated by that teloblast (small red cells making a bandlet). At the top is shown the front end of a mature leech (cut off from the rest of the body, which is not shown) showing dye-labelled cells descending from the injected teloblast in the segmental ganglia on the injected side (red shading indicates collections of labelled cells).

1.4.3 Other invertebrates

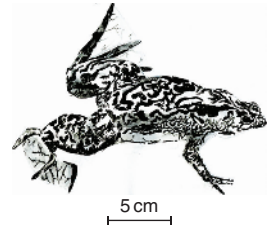
Other invertebrates have been used as model organisms for research on neural development, including sea urchins (used since the 1800s because their embryos are easily viewed under the microscope), leeches (Box 1.3) and sea squirts (which, despite their appearance, are most closely related to vertebrates). These species have provided invaluable insights and have significant advantages for some studies. Sea squirts, for example, will be discussed again in the context of neural induction in Chapter 3.

1.5 Vertebrate model organisms

1.5.1 Frog

Among vertebrate model organisms, the African clawed frog *Xenopus laevis* (right) provided some of the earliest and most important insights into mechanisms of embryogenesis, including the initial formation of the nervous system. Starting in the late 1800s, German scientists exploited the relatively large robust eggs of frogs, and of other amphibians, in experiments aimed at understanding how specific groups of cells instruct other groups of cells to develop in particular ways. They studied the extent to which specific groups of cells are committed to the fates they are normally instructed to follow. At the heart of this work was the question: could the normal developmental fates of cells be altered by experimental manipulation? The experiments involved microsurgery on the embryos, which are easily accessible since they develop outside the body. In some experiments, portions of embryos were grafted from one region into another, to discover how they develop at the new site and their effects on their new neighbours. In other experiments, cells were cultured in isolation. One scientist, Hans Spemann, had his great contribution to this field of experimental embryology recognized by the award of a Nobel Prize in 1935.⁹ The discoveries that were made will be discussed in Chapter 3.

Unfortunately, *Xenopus laevis* is not ideal for forward genetics because it takes many months for females to reach maturity, which would make the breeding required to establish mutant lines difficult. They also have four copies of many genes (allotetraploid), complicating the study of inheritance. The feasibility is greater with *Xenopus tropicalis*, which matures more quickly and is **diploid**. In reverse genetic experiments, the size, accessibility and robustness of *Xenopus* eggs and embryos does make them favourable targets for the injection of molecules designed to raise or lower levels of specific gene products. The levels of a specific protein can be raised by injecting mRNA molecules; the levels of specific proteins can be lowered by injecting molecules that interfere with the function of specific mRNAs (this is sometimes called a knockdown; see Figure 1.4).



diploid an organism with a pair of each type of chromosome.

⁹www.nobelprize.org/nobel_prizes/medicine/laureates/1935/spemann-lecture.html [20 November 2010].

1.5.2 Chick



Chick (*Gallus gallus*) embryos are favoured model organisms because of the ease with which eggs can be obtained and stored. Furthermore, the embryo has a short incubation time; the nervous system is well developed after only a few days; and it is relatively easy to observe and manipulate the embryo by opening a small window in the shell. Since the early 1900s experimenters used fine surgical methods (micromanipulation) to transplant pieces of live embryos from one place to another (a process known as grafting) to find out how the transplanted parts respond (discussed further in Chapter 3). More recently, chick embryos have proved useful models for testing the functions of developmentally important genes using mRNA-mediated reverse genetic methods (Figure 1.4).



1.5.3 Zebrafish



In the past decades the small freshwater zebrafish (*Danio rerio*, left), native to India, has become another very popular vertebrate model organism. Since its eggs are fertilized and its embryos develop externally, they are readily accessible for experimental manipulation. Its embryos develop rapidly and are translucent, allowing morphogenesis to be visualized and recorded relatively easily. The process can be observed under the microscope in real-time, as it unfolds (Figure 1.5), using stains that are compatible with life such as **green fluorescent protein** and its variants (Box 1.4). Not only are reverse genetic approaches being exploited successfully in zebrafish, but the species is also proving suitable for large-scale forward genetic screens in which mutagens are used to create lines of fish carrying phenotypic abnormalities (along the lines shown in Box 1.1).

1.5.4 Mouse



Among mammalian species, the mouse (*Mus musculus*) has tremendous advantages for molecular genetics. Originally, Gregor Mendel studied inheritance in mice, but his work was stopped by the religious hierarchy in Austria who considered it inappropriate for a monk to share a room with copulating animals! Mouse inheritance was re-examined at the start of the

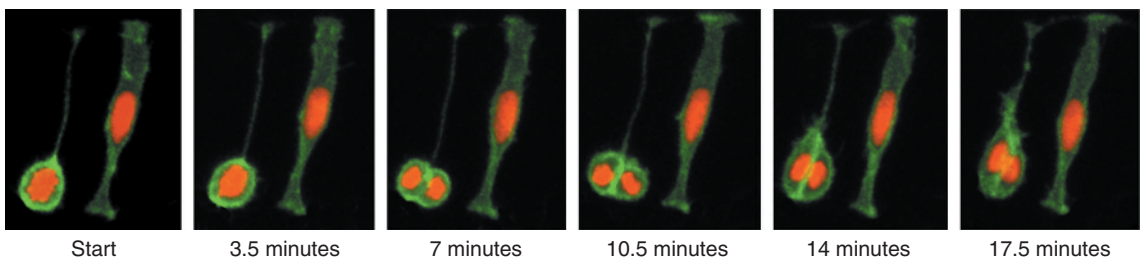
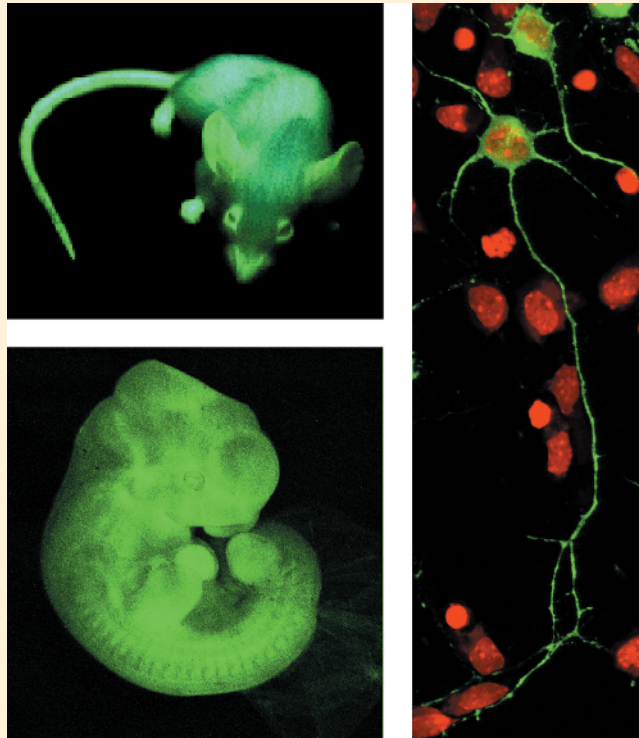


Figure 1.5 A time-lapse series of images of labelled neural cells in a live zebrafish embryo showing one dividing while the other remains quiescent, made by Paula Alexandre in Jon Clarke's laboratory, King's College London, UK. Green is green fluorescent protein (GFP) that is being used to label cell membranes. Red is red fluorescent protein (RFP), a different fluorescent protein that is being used here to show nuclei (see Box 1.4 for details of these fluorescent molecules).

Box 1.4 Green fluorescent protein (GFP)



GFP in living cells fluoresces bright green when illuminated with blue light. It was first isolated from jellyfish. Its gene can be introduced into organisms in a variety of ways, so as to label either all their cells (e.g. the mouse pictured top left or a mouse embryo pictured bottom left) or only some of their cells (e.g. pictured right: red is a non-specific stain for all nuclei). Whether all cells are labelled or only some specific cells are labelled depends on what regulatory sequence the experimenter chooses to activate the GFP gene. GFP can also be joined to specific proteins to visualize their subcellular location. Labelling cells with GFP can be used in numerous ways, for example to follow cell lineages (Box 1.3; see also Section 7.2 in Chapter 7) or to study where and when regulatory elements activate their genes. We will describe many examples of the use of GFP throughout the book. Martin Chalfie, Osamu Shimomura and Roger Y. Tsien were awarded the Nobel Prize in chemistry in 2008 for their discovery and development of GFP.¹⁰ Variations of GFP that fluoresce with other colours are now available, allowing more than one label to be used simultaneously (Figure 1.5). Photographs of GFP-labelled embryo and cells are courtesy of Tom Pratt, University of Edinburgh, UK; photograph of GFP-labelled adult mouse is reprinted from Hadjantonakis, A.-K. *et al.* (1998) Generating green fluorescent mice by germline transmission of green fluorescent ES cells. *Mechanisms of Development*, **76**, 79–90, with permission from Elsevier.

¹⁰http://nobelprize.org/nobel_prizes/chemistry/laureates/2008/chalfie-lecture.html [20 November 2010].

stem cell a relatively unspecialized cell that can divide repeatedly to regenerate itself (self-renewal) and can give rise to more specialized cells, such as neurons or glia.

homologous recombination a phenomenon in which nucleotide sequences are exchanged between two similar or identical strands of DNA.

CRISPR/Cas9 a naturally occurring defensive system used by bacteria that has been adapted for experimental use in any species to allow targeted, highly efficient alterations of genome sequence.

genotype the genetic make-up of a cell or organism.

twentieth century in France by Lucien Cuénot, who confirmed Mendel's predictions from plants. Many inbred strains and lines selected for particular phenotypes now exist and are maintained for experimental research by large breeding facilities around the world, such as the Jackson Laboratories in the USA and the RIKEN BioResource Centre in Japan.¹¹ Some lines have come from screening for abnormal phenotypes among populations of laboratory mice in which mutations have occurred spontaneously or been induced randomly with mutagens (e.g. the chemical *N*-ethyl-*N*-nitrosourea, or ENU).¹² For many lines the genes and their variants responsible for the phenotypes have been identified. This is an example of forward genetics (Box 1.1), but in many cases the lines are derived from reverse genetic approaches made possible by a great breakthrough in the 1980s.

The breakthrough came from the discovery by Martin Evans, Matthew Kaufman and Gail Martin that **stem cells** from the early mouse embryo could be grown in culture. When reintroduced into mouse embryos, these cells are able to generate all of the cell types in the body, a property known as **pluripotency** (Figure 1.6). The genomes of these stem cells, known as **embryonic stem cells** (or **ES cells**), can be manipulated by adding DNA sequences, for example encoding specific proteins, or mutating genes by replacing endogenous DNA sequences with mutated sequences by **homologous recombination** (Figure 1.7). The manipulated ES cells can then be used to make genetically engineered mice, as we will explain in a moment. However, first we should expand briefly on the DNA manipulation step.

Nowadays, most researchers would use newly developed methods such as the **CRISPR/Cas9** system to improve the efficiency of the genetic changes in the ES cells (Figure 1.7). The term CRISPR was coined in the 1980s to describe short DNA sequences that bacteria use to make RNA that can defend them against viruses by cutting the viral DNA. For what it is worth, CRISPR (pronounced 'crisper') stands for Clustered Regularly-Interspaced Short Palindromic Repeats (the abbreviation might be easier to remember if you think of CRISPR making the DNA 'toast!'). Cas stands for CRISPR-associated protein: it is the enzyme attached to the defensive RNA molecules that actually cuts the DNA. Scientists have extended this naturally occurring system to make cuts at specific places in DNA. You can find more on the details of how CRISPR/Cas9 works elsewhere¹³ (and see Figure 1.7). One important point to appreciate is that, unlike traditional methods that could only be used efficiently in mice, CRISPR/Cas9 has been used effectively in a wide range of other vertebrates and invertebrates.

Once modified, by whatever method, ES cells can then be injected into early mouse embryos to generate **chimeras**: a chimera is an individual created when cells of different **genotypes** come together to form an embryo (Box 1.5). In these chimeras, some of the cells derived from ES cells with

¹¹ <http://www.jax.org/> [20 November 2010] and <http://mus.brc.riken.jp/en/>.

¹² For more details of these screening methods in mice, see Kile, B.T. and Hilton, D.J. (2005) The art and design of genetic screens: mouse. *Nature Reviews Genetics*, 6, 557–567.

¹³ Sander, J.D. and Joung, J.K. (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*, 32, 347–355.

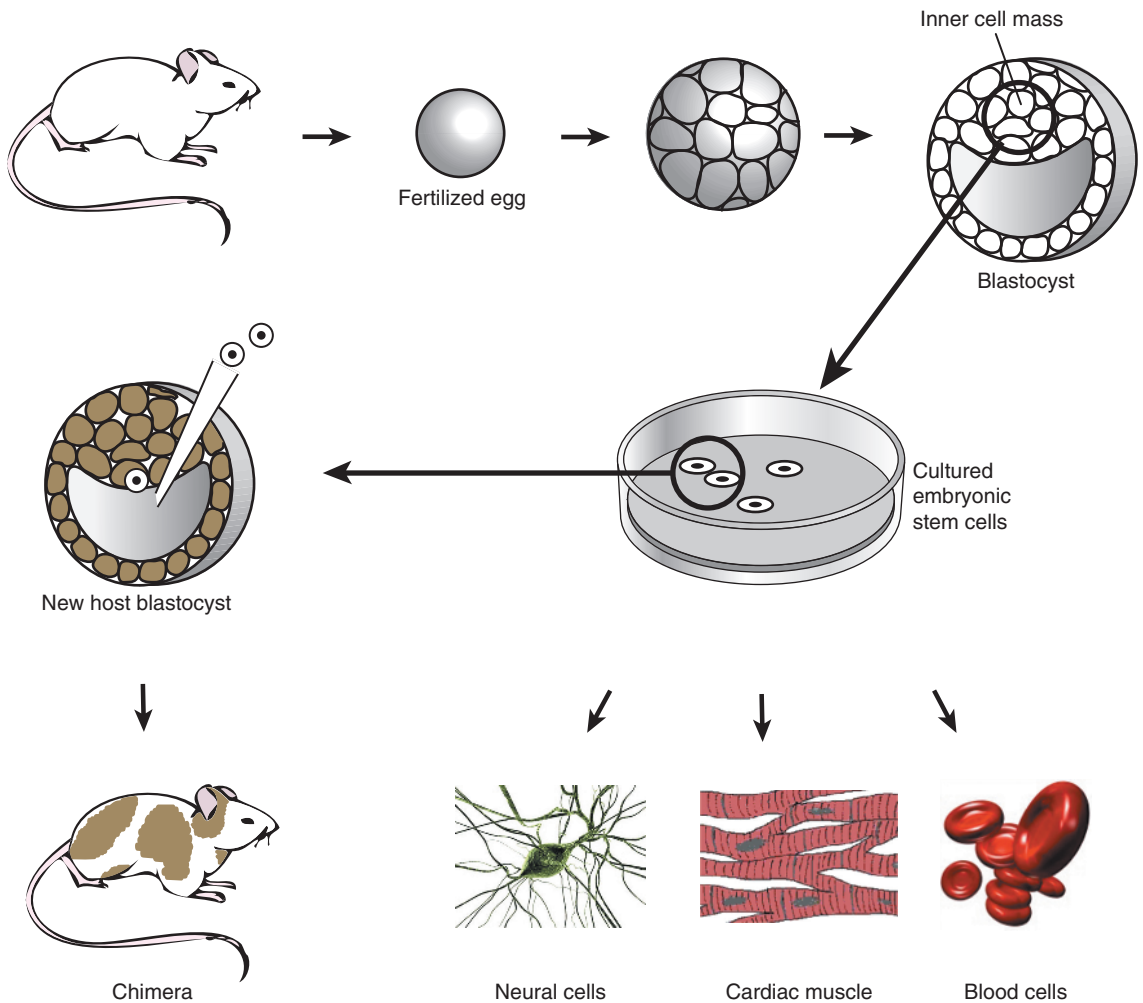


Figure 1.6 Mouse embryonic stem (ES) cells are derived from the inner cell mass of a mouse blastocyst (here from a line of mice with a white coat). The inner cell mass is transferred into a culture medium in a plastic laboratory culture dish. The cells from the inner cell mass divide and spread over the surface of the dish to become ES cells. ES cells can be differentiated into many cell types in culture, including neurons, heart muscle cells and blood cells. They can be injected into new blastocysts, here from a line of mice with brown fur (although the blastocyst cells are shaded brown to help make the diagram clear, in reality they would be no different in colour to those from the blastocyst of mice with white fur). The injected blastocysts are implanted into the uterus to generate chimeric (Box 1.5) offspring comprising a mixture of cells derived from the ES cells and the host blastocyst (the chimeric mice would have a mixture of brown and white fur).

modified genomes will form germ cells and therefore genetic alterations made in the ES cells can be transmitted through the germ line to subsequent generations (Figure 1.7). In this way, hundreds of lines of **transgenic** mice with specific additional DNA sequences (known as knock-in mice) or with loss-of-function mutations in specific genes (known as knock-out mice) have been established and studied. The generation of knock-out mice is an excellent example of reverse genetics. It was particularly effective in advancing an understanding of the mechanisms of neural development. The homologues of many developmentally important genes discovered initially in

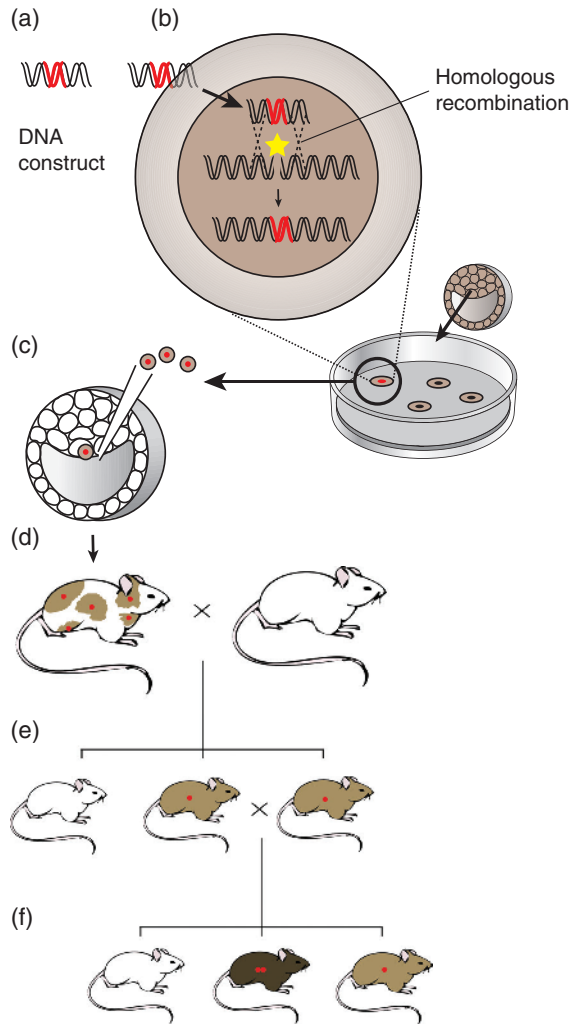


Figure 1.7 Reverse genetics: generation of transgenic mice. This method allows the experimenter to manipulate specific genes so as to learn about their functions. For example, a normal gene might be replaced with a modified version to generate a knock-out, or we might want to insert sequences to make conditional mutants (Figure 1.8). To do this, the genome of embryonic stem cells (see Figure 1.6) is manipulated in culture. (a) The experimenter constructs DNA molecules that have (i) stretches at each end identical to sequences in and/or around the gene that is to be mutated and (ii) a central portion whose incorporation into the target gene will prevent its function (red). (b) To enable the embryonic stem cells to take up these DNA molecules, they are put into the solution around the embryonic stem cells and a current is passed through the cells (this is called electroporation). In some cells the flanking sequences swap places with the identical sequences in the genome (a chance event called homologous recombination, indicated in (b) by the two crossed broken lines), carrying the central portion into the genome to prevent the function of the target gene. Nowadays, experimenters would greatly increase the efficiency of this process by using the CRISPR/Cas9 system to induce a break at a specific place in the targeted region of the DNA (yellow star). This is done by giving the cells RNA molecules linked to an enzyme called Cas9 nuclease that cuts DNA (not shown in the diagram). The sequence of the RNA is chosen by the investigator to guide it to the correct place in the DNA and so these RNAs are known as guide RNAs. If all the experimenters want is to disrupt the gene and not replace anything, they might not bother electroporating the new DNA molecules and just induce a break in their target, because when it heals it will usually do so imperfectly, leaving behind a disruptive mutation. (c) The mutated embryonic stem cells are then injected into blastocysts to generate chimeras (d) in which some of the animal's cells are mutant, including some germ cells. (e) Since some germ cells in these chimeras should be mutant, subsequent breeding with normal mice will generate offspring in which all cells are heterozygous for the mutation as well as other mice that are normal. (f) A second round of breeding between heterozygotes will generate some mice that are homozygous for the mutation (double red dot), some that are heterozygous for it (single red dot) and some that are normal. Many variations of this method are possible: for example, DNA containing an entire gene controlled by appropriate regulatory elements might be added to the genome so as to overproduce a specific protein in a specific part of the animal.

Box 1.5 Chimeras and mosaics

Chimeras and mosaics are animals that have more than one genetically distinct population of cells. The terms mean different things but are sometimes used incorrectly. *Chimeras* arise when cells originating from different fertilized eggs come together to create a single embryo. Chimeras can be created by ES cell injections (Figures 1.6 and 1.7) or in other ways, for example by pushing two very early embryos together so that they fuse. In *mosaic* organisms the genetically distinct cell types all arise from a single fertilized egg. For example, normal female mammals are mosaic. They have two X chromosomes but, early in embryogenesis, the vast majority of genes on one X chromosome are functionally inactivated, a process called X chromosome inactivation. This inactivation occurs randomly: in roughly half of a female's cells, the paternal X chromosome is inactive and in the other half the maternal X chromosome is inactive. This has important biological and medical implications, particularly for X-linked genetic diseases such as Fragile X Syndrome and Rett Syndrome (see Chapter 12, Box 12.3).

Drosophila were targeted and shown to play critical roles in the development of the mammalian central nervous system. Work developing the methods that made this possible was recognized in 2007 by the award of a Nobel Prize to Mario Capecchi, Martin Evans and Oliver Smithies.¹⁴

Since the introduction of methods for the generation of transgenic mice in which *all* cells in the organism are mutated (sometimes referred to as **constitutive mutants**), other approaches have been developed to target the DNA of only a specific subset of cells, or to induce mutations at a selected time in development, or both. Such mice are mosaic (Box 1.5) and are usually called **conditional transgenics**. One advantage of this approach is that it allows the experimenter to focus on the effects of disrupting a gene only in specific cells of interest. It minimizes complications arising from the possibility that, if all the organism's cells are mutated, the cells of interest will also be affected by secondary, collateral consequences of the gene's loss in other regions. The conditional transgenic approach can also be used to mark specific cells early in development so that their progeny can be followed in subsequent development. This is an elaborate form of lineage tracing (Box 1.3) that we shall return to in Chapter 7 (see Box 7.2). One well-established way of making these types of conditional transgenic mice uses the **cre-loxP** system (Figure 1.8). In this method, two separate lines of transgenic mice are generated using the techniques described in the previous paragraph. We make one line that produces a **bacteriophage** protein, cre recombinase (cre stands for 'causes recombination'; this protein is never normally made in mice), *only* in the cells that we want to mutate. We modify the genome of the other line so that the sequence we want to remove is flanked by sequences called *loxP* sites (also from bacteriophage) that are too short to interfere with the function of the surrounding DNA. When cre recombinase gains access to these *loxP* sites in mice produced by crossing the two lines, it will join the *loxP* sites together, removing the DNA between them.

conditional transgenic animal in which only some cells in the organism are mutated.

¹⁴http://nobelprize.org/nobel_prizes/medicine/laureates/2007/evans-lecture.html [20 November 2010].

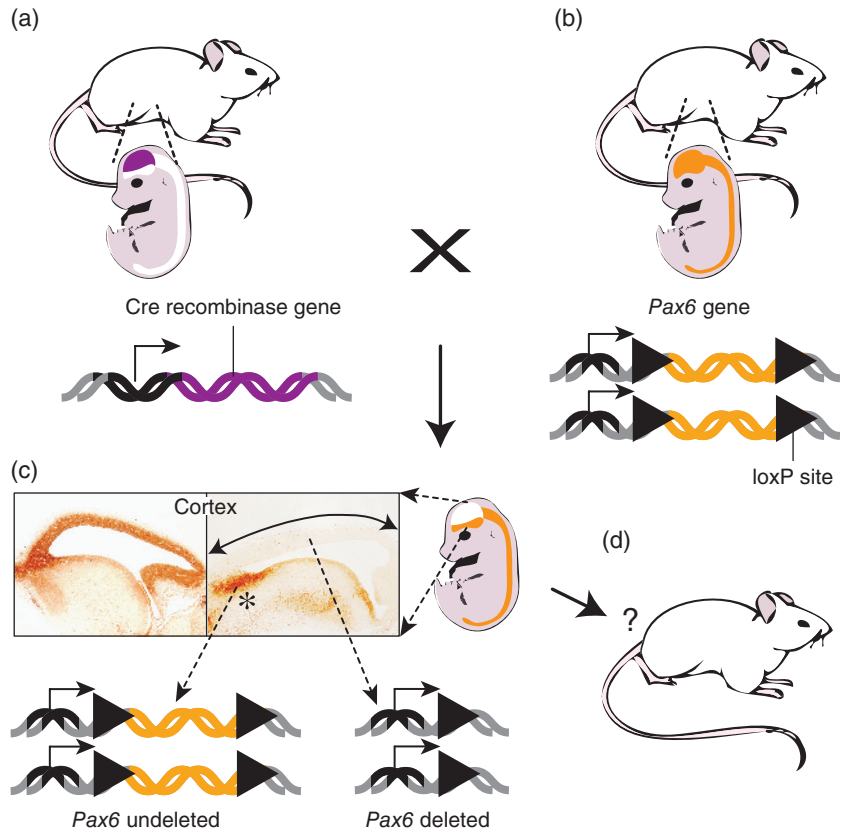


Figure 1.8 Reverse genetics: generation of conditional transgenic mice with the cre-loxP system. (a) An example of a transgenic mouse that has been made to produce the bacteriophage enzyme cre recombinase specifically in its cerebral cortex when it was an embryo (purple). To achieve this, the cre recombinase gene is controlled by regulatory sequences in the DNA that are known, from previous work, to operate only in the cerebral cortex (regulatory sequences have right-angled arrows above them, which is a common convention; they are discussed further in Chapter 3). (b) An example of a transgenic mouse that has loxP sites (triangles, again a common convention) flanking both copies of a gene that is present throughout the nervous system of the embryo; in this case the gene is called *Pax6* (orange: it codes for a protein that is a **transcription factor**; you can find more about these molecules in Chapter 3). (c) In the offspring produced by breeding these two lines of mice together, *only* those cells that make cre recombinase (i.e. cerebral cortical cells) will delete the target gene. In the photographs of thin slices of the brain, *Pax6* (which is stained brown using a method called immunocytochemistry, described in Chapter 3, Box 3.4) is deleted from the cerebral cortex of the conditional mutant. The cortex is the tissue underneath the double-headed arrows. The mutant is on the right and a normal embryo is shown on the left for comparison. *Pax6* is not affected in the regions beneath the cortex (asterisk). (d) Sometimes one finds that conditional mutants survive longer than mice in which all cells are affected by a mutation, extending the period over which the effects of mutation can be studied. This depends on what the mutated gene does. (You can find an example of how the cre-loxP can be put to other uses in Chapter 7, see Box 7.2.)

Although the nervous system of mice is very much smaller than that of humans, it does share many of the same major structures carrying out similar functions. The mouse is, therefore, a popular choice of model organism for researchers wishing to investigate the molecular mechanisms of development of neural structures found in humans. It breeds relatively quickly and easily with large numbers of offspring and it is genetically tractable.

Similarities between mice and humans extend to the DNA level: a large multinational study reported in 2002 that 99% of mouse genes have homologues in humans and 96% of genes in the two species are arranged on the chromosomes in the same order. This degree of similarity is remarkable. While it does help justify the use of the mouse as a model in which to learn more about human development, it also raises an intriguing unanswered question: what in our DNA makes us so different from mice (Box 1.6)?



1.5.5 Humans

Much of our understanding of how humans develop is based on extrapolation from knowledge gleaned using non-human model organisms including those described above. It would be a mistake, however, to imagine that there is little we can do to study our own development more directly. Certainly, it is possible to examine human embryos using **magnetic resonance imaging (MRI)** and to investigate their structure and molecular constitution in great detail in specimens obtained post-mortem. Although it is more complicated to get embryonic or fetal material from humans than from other species, many of the issues can be minimized by using material from collections called **biobanks**; you can visit the website of one such biobank here.¹⁵

If we want to go beyond simply observing human development to investigating the underlying mechanisms, options are more limited than in other species, for obvious ethical reasons. While we cannot manipulate human embryos or their genomes for experimental purposes, many researchers study the consequences of naturally occurring mutations with a view to better understanding the genetic basis of disease. In fact, major contributions to developmental neurobiology in general have been made by identifying genes whose disruption in humans is associated with disease. Examples will be found throughout later chapters.¹⁶ In overview, this approach can be seen as an excellent example of forward genetics, working from phenotype to gene: one starts from patients with genetic disorders and discovers the underlying genomic defects. This approach has been greatly enhanced by advances in sequencing technologies (Box 1.6). These methods allow us to sequence entire genomes efficiently and also to discover the sequences of all of the mRNA molecules made in cells of interest (known as their **transcriptomes** – remember that, unlike genomes, transcriptomes vary greatly between cells of different types and it is this variation that makes cells different). These methods have revolutionized our understanding of human biology, but they are equally applicable to any other species.

Can we take a reverse genetics approach to studying human development? To do so, we would need to start with a gene (or genes) whose function we want to understand and carry out genetic manipulations, such as deletions. In Box 1.7 you can read about recent advances in stem cell technologies that are offering solutions that avoid unacceptable experimentation on living embryos.

magnetic resonance imaging (MRI) a technique using strong magnetic fields to make images of the body's internal structure and function.

biobank a repository (usually organized and funded at a national or international level) that stores biological samples (usually human) for use in research.

¹⁵ <http://www.hdbr.org/>.

¹⁶ For further reading, we suggest the book by Strachan, T., Goodship, P. and Chinnery, P. (2014) *Genetics and Genomics in Medicine*, Garland Science.

Box 1.6 Nucleic acid sequencing and the genetics of humanness

The sequence of the four bases that make up DNA – adenine (A), guanine (G), cytosine (C) and thymine (T) – encodes the blueprint of an organism, called its **genome**. Methods for determining the order of these bases (a process called **sequencing**) in short stretches of DNA were invented in the 1970s. Techniques for rapid, efficient sequencing of the billions of bases in the entire genome of an organism were invented in the 1990s and are referred to as **next-generation sequencing** methods.¹⁷ Sequencing the genome of an organism allows us to read the entire genetic blueprint required to make an organism. Sequencing the genomes of an increasing number of species, including humans, has exposed similarities and differences in their genotypes that might explain similarities and differences in their phenotypes. One general conclusion is that remarkably little of the genome codes for proteins (<2% in humans) and many proteins are very similar in species that are otherwise very different. For example, our proteins are 99% identical to those of chimpanzees and, on average, about 85% identical to those of mice. Researchers have started to look for critical differences in the vast tracts of the genome between the sequences that code for proteins. These mysterious regions are sometimes referred to by the pejorative term 'junk DNA' and they show relatively greater interspecies variation than protein-coding sequences. Many of them code for several types of RNA that can influence the protein-coding genes, for example **microRNAs**, which are discussed in Chapter 3 (Section 3.8.4). The evolution of differences in some of these non-coding regions, which has occurred particularly rapidly in humans (in genomic regions called Human Accelerated Regions), might have made a major contribution to evolutionary change and might help explain what, from a genetic perspective, makes us human. You can read more on this topic here.¹⁸ The methods can also be used to sequence RNA molecules to determine the transcriptomes of cells of interest. The methods are now sufficiently advanced to allow sequencing of nucleic acids from single cells.

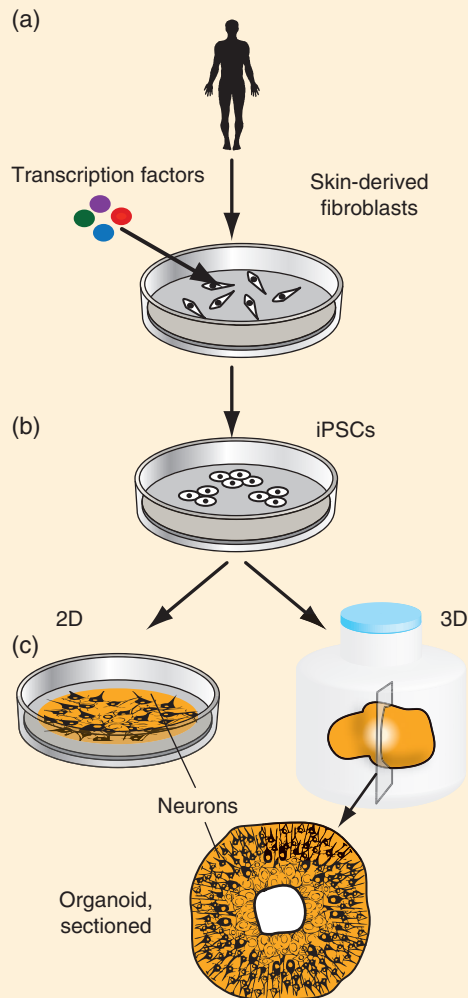
1.5.6 Other vertebrates



Many other mammalian species have been used to study specific aspects of development, for example cerebral cortical development and the development of vision. Prominent among them are the ferret, cat and monkey. They do not have the advantages of the mouse for molecular genetics, although the development of the CRISPR/Cas9 method for genetic manipulation (Section 1.5.4 and Figure 1.7), which appears to be applicable in any species, has been opening new opportunities in this regard. These species do, however, offer considerable advantages for some types of study. For example, ferrets are born more immature than many other mammalian species commonly used in research, allowing easier access to the developing nervous system at an earlier stage. Cats, unlike mice, have relatively high resolution binocular vision and have been subjects of research on the development of the visual system for many decades; the success of research in this area was

¹⁷ Goodwin, S. *et al.* (2016) Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, 17, 333–351; Bras, J. *et al.* (2012) Use of next-generation sequencing and other whole-genome strategies to dissect neurological disease. *Nature Reviews Neuroscience*, 13, 453–464.

¹⁸ Pratt, T. and Price, D.J. (2016) Junk DNA used in cerebral cortical evolution. *Neuron*, 90, 1141–1143.

Box 1.7 Using stem cells to model human development


Induced **pluripotent** stem cells (iPSCs) are a new type of stem cell, first described in 2006. They can be made from humans and other species, such as mice. The figure illustrates a common method for their generation. (a) **Somatic cells**, such as skin cells, are taken from a donor and placed in culture. Then four **transcription factors** – cMYC, OCT4, KLF4 and SOX2 – are added to the cultured cells. You can find out more about transcription factors, including some of those mentioned here, in Chapter 3. (b) Remarkably, adding just these four transcription factors is enough to convert fully differentiated cells into cells with properties very similar to ES cells (Figure 1.6). iPSCs show the key hallmarks of pluripotent stem cells. They can divide indefinitely in culture and are able to give rise to a wide variety of differentiated

pluripotency the ability of a cell (e.g. an embryonic stem cell or an iPSC) to differentiate into many or all cell types in the body.

somatic cell any cell of an organism with the exception of the reproductive cells, or gametes.

transcription factors proteins that bind to DNA to regulate gene transcription.

(Continued)

Box 1.7 (Continued)

progeny, including neurons. It is relatively straightforward to manipulate their genome using CRISPR/Cas9 (Section 1.5.4), for example to inactivate specific genes or to add genetic material, allowing us to experiment on models of the developing human brain. (c) Like ES cells, cultured iPSCs can be differentiated into neurons in flat, two-dimensional (2D) cultures. More recently, iPSCs have been used to generate **cerebral organoids**¹⁹, which are 3D structures that resemble embryonic brains (in fact, they are sometimes called minibrains). iPSCs and organoids offer great potential as tools to study brain development. We can also make iPSCs and cerebral organoids directly from patients with neurodevelopmental disorders that have a genetic basis, including **schizophrenia**, **autism** and intellectual disability. This will allow us to study the genetic basis of these disorders, since patient-specific iPSCs have the same genetic make-up as the person from whom they were derived. For example, a recent study generated iPSC-derived forebrain neurons from patients with Rett syndrome, a neurodevelopmental disorder caused by mutations in a gene called *MECP2* that manifests itself in young children aged 6–18 months and is characterized by intellectual disability and autism-like symptoms.²⁰ (See Chapters 11 and 12, Boxes 11.4 and 12.3 for more discussion on these conditions.) Compared to iPSC-derived neurons from healthy people, those from patients showed a delay in the development of inhibitory networks (see Chapter 11 for a discussion of inhibitory neurons). We can then work on these patient-derived iPSCs, attempting to correct their defects. This type of research is likely to offer new insights that might eventually lead to new treatments.²¹

schizophrenia a mental disorder characterized by abnormal behaviour and failure to understand reality.

autism a mental disorder characterized by difficulty in social, verbal and non-verbal communication and repetitive behaviours.

recognized by the award of a Nobel Prize to David Hubel and Torsten Wiesel in 1981²² (see also Box 10.5 in Chapter 10). Monkeys have the advantage of being closely related to humans. They share many primate-specific specializations that cannot be studied in other species. They have been used particularly to study the development of neural structures, connections and functions at high levels of the nervous system. The amount of research done with rodents and invertebrates is far more than that with higher species, which are costly to maintain, slow to breed and whose use needs strong ethical justification. Research on these species will be discussed mainly in the book's final chapters.

¹⁹For a review on this topic, see Mason, J.O. and Price, D.J. (2016) Building brains in a dish: prospects for growing cerebral organoids from stem cells. *Neuroscience*, 334, 105–118.

²⁰Tang, X. *et al.* (2016) KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome. *Proc. Natl Acad. Sci. USA*, 113, 751–756; Chailangkarn, T. *et al.* (2012) Modelling neurodevelopmental disorders using human neurons. *Current Opinion in Neurobiology*, 22, 785–790.

²¹To read more about how research using iPSCs could transform our understanding of human genetic disorders, see Hockemeyer, D. and Jaenisch, R. (2016) Induced pluripotent stem cells meet genome editing. *Cell Stem Cell*, 18, 573–586.

²²http://nobelprize.org/nobel_prizes/medicine/laureates/1981/wiesel-lecture.html [20 November 2010].

1.6 Observation and experiment: methods for studying neural development

Biological research often progresses through the following stages: (i) naturally occurring phenomena are observed; (ii) experiments are designed to test hypotheses about the mechanisms responsible for the phenomena; (iii) the experiments are carried out; (iv) hypotheses are refined, dependent upon the outcome of the experiments. To discover the mechanisms responsible for a phenomenon, it is often necessary to challenge the biological system by altering some aspect of it and assessing the effects. In studying neural development, for example, a commonly used approach is to remove a specific gene's function by making a line of knock-out mice to discover whether that gene is *necessary* for the developmental phenomenon in question. Alternatively, one might cause a gene to become active in the wrong place so as to discover whether, in those cells, its activity is *sufficient* to cause the developmental phenomenon or whether other factors are important. Similarly, a brain region might be removed or manipulated. These and other similar approaches are critically dependent on methods for manipulating developing cells and their environment. In this chapter we have described how several model organisms offer advantages for experimental interventions that alter gene function, protein production and cellular environments (e.g. by transplanting cells).

In addition, experimental biology relies on methods for observing biological phenomena and for assessing the effects of experimental manipulations. As technology has advanced scientists have been able to employ an ever-increasing range of sophisticated molecular, cellular, anatomical and functional methods to observe when and where specific genes act during development, to observe cells as they proliferate, grow, migrate, differentiate and die, and to observe cells functioning physiologically. Many such observational methods will be explained at appropriate places throughout the rest of this book.

Finally, there is the important issue of deciding which experiments are most likely to give insights into the mechanisms of development. An approach that can help greatly with this involves the use of formal computational models of developing systems. The design of formal models to test hypotheses relating to specific biological questions is now common to many areas of biology. Such models are formulated as a set of mathematical equations that represent the actions of the cellular or subcellular elements and their interactions in the biological system under consideration. Solution of the equations, which often uses computer simulation, specifies how (according to the model) the systems under consideration will behave under given conditions. This allows theoretical predictions to be made that can then be tested experimentally.

All biological research is based on the development of hypotheses but often these hypotheses are expressed in informal terms, using words or diagrams to represent the idea. Formal models are simply an extreme version of this same process in which the use of mathematics forces the designer to make a logically consistent hypothesis. One advantage of this approach is that it can generate theoretical reasons against hypotheses that might seem perfectly plausible at a less formal level. Another advantage is that formal



models can involve a larger number of interacting elements than can be accommodated easily in informal models. Formal models do have potential problems, of course. They have to make assumptions concerning the underlying biology they are designed to model and in some cases the assumptions they are based on are questionable, or the model might be too simple. In the worst case it might not be possible to test the conclusions from a formal model. The development of formal models is not always possible – it depends on the system and the experimental questions being asked – but where their application is feasible they can provide an invaluable guide to experimental research. This will be discussed again particularly in Chapter 10 (Section 10.3).

1.7 Summary

- Understanding how nervous systems develop remains a major challenge for research with implications for human health and future technologies.
- Most modern developmental neurobiology seeks to understand the molecular mechanisms controlling key developmental events.
- The development of methods for efficiently sequencing entire genomes and transcriptomes and for manipulating the functions of genes in increasingly specific ways in a broader and broader range of species has had a massive impact in this field.
- A small handful of animal species, referred to as model organisms, are used in most developmental neurobiological research because each has clear advantages for certain types of research. Such organisms include flies, worms, frogs, fish, chicks, mice and humans.
- To understand the molecular genetics of development two broad approaches are used: (i) forward genetics, where one seeks to find the genes responsible for a particular aspect of an organism's phenotype; (ii) reverse genetics, where one starts with a gene and manipulates it to discover its functions.
- Some areas of developmental neurobiology are amenable to the application of increasingly powerful computational modelling approaches to test hypotheses theoretically prior to the use of experimental methods.