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. Fig. 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 are 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 human history, during which growth in population size and sophistication have emerged hand-in-hand, each stage building on what went before – with one obvious difference that development repeats over and over again in the same way in each species. 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.

![Figure 1.1: A node of Ranvier](image-url)
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 where the limits of our knowledge are, since it is at least as important and interesting to acknowledge what we don’t 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 100,000,000,000 cells are generated with about 100,000,000,000,000 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 don’t 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 don’t 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 development 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, autism and epilepsy (Fig. 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. Turning to possible treatments, it has been suggested that brains suffering from neurodegenerative diseases might be repaired by implanting new cells into the nervous system. Such an approach would require that implanted cells 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.

### 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. 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

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1 For a comprehensive list and images of the numerous developmental and genetic diseases that affect humans, see www.gfmer.ch/genetic_diseases_v2/index.php [20 November 2010].
international organizations are taking it seriously enough to put large amounts of money into research aimed at establishing whether it might be possible.

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 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* (left), 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 (Fig. 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 13 000–14 000 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 to study human disease\(^2\): 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.\(^3\)

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.\(^4\) 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 Fig. 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...
Box 1.1  Forward genetics: working from phenotype to gene

This diagram in Box 1.1 shows an example of a strategy in *Drosophila* to mutate randomly a large number of genes and then screen for those mutations that produce abnormal phenotypes in the offspring. This allows the experimenter to go on in further work to identify genes that are important for the normal generation of the structures that are rendered defective. 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 are crossed to wild-type females (top left) to generate 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 between the members of each of 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 that generate phenotypes of interest. Similar approaches can be taken 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 (Section 1.5.4). 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.

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5 The 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.

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) To make the first line, the experimenter generates a length of DNA with three components: (i) the sequence of the gene (X) to be activated, (ii) a sequence that will activate the gene (called the Upstream Activation Sequence, or UAS) provided it is bound by a protein called GAL4 and (iii) a sequence called a P-element (not shown) that allows the whole piece of DNA to enter the genome when it is injected into an embryo. In this way, the experimenter generates a line of transgenic organisms (called a responder line) carrying this part of the system. However, gene X will not be activated in this line since GAL4, which the UAS needs if it is to activate gene X, is a yeast protein that would not normally be there. Thus, the second part of the GAL4/UAS system is designed to deliver GAL4 to cells where the experimenter wants the gene of interest (X) to be activated.

(2) For the second line, the experimenter generates another line of transgenic organisms (called the driver line) in which GAL4 is activated selectively in the cells where the experimenter eventually wants the gene of interest (X) to be activated. To do this, the experimenter makes 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 P-element (not shown) to carry the DNA into the genome. Making this piece of DNA requires the experimenter to select a 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.

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.
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 descendents can help define cell lineages; suitable markers include dyes or reporter molecules (for example green fluorescent protein, see Box 1.4) 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 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.

Bottom: 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). Top: front end cut off from a mature leech showing dye labelled cells descended from the injected teloblast in the segmental ganglia on the injected side.
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 specific genes (for example, using RNA interference methods, Fig. 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.\(^7\) 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 11).

### 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 frog, in particular 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 frog eggs and embryos in experiments aimed at understanding how specific groups of cells instruct others to develop in particular ways. In this work the scientists 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 the field of experimental

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**hermaphrodite** an organism with both male and female sexual characteristics and organs.
embryology through research on *Xenopus* recognized by the award of a Nobel Prize in 1935;⁸ the discoveries that were made will be discussed further 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, and they 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 knock-down, Fig. 1.4).

### 1.5.2 Chick

Chick (*Gallus gallus*) embryos are favoured model organisms because of the ease with which eggs can be obtained and stored. The embryo has a short incubation time – the nervous system is well developed after only a few days – and is very accessible, allowing easy observation of embryogenesis. 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 (Fig. 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 accessible for experimental manipulation. Its embryos develop rapidly and are translucent allowing morphogenesis to be recorded relatively easily as it unfolds under the microscope (Fig. 1.5) using stains such as **green fluorescent protein** and its variants that are compatible with life (see 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).

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Fig. 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* 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).
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 this was stopped by the religious hierarchy in Austria who considered it inappropriate for a monk to share a room with copulating animals! The topic was re-examined at the start of the 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.9

For many lines the genes and their variants responsible for the phenotypes have been identified (an example of forward genetics). These lines are often the results of 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).10

A huge breakthrough came in the 1980s with the discovery by Martin Evans, Matthew Kaufman and Gail Martin that stem cells from the early mouse embryo could be grown in culture and when reintroduced into mouse embryos were able to generate at least most if not all of the cell types in the body, a property known as pluripotency (Fig. 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 (Fig. 1.7). The modified 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 modified genomes will form germ cells and therefore genetic alterations made in the ES cells can

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stem cell a relatively unspecialized cell that can divide repeatedly to regenerate itself (self-renewal) and 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.

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9 http://www.jax.org/ [20 November 2010].
be transmitted through the germ line to subsequent generations (Fig. 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 since the homologues of many developmentally important genes have been knocked out in mice.

Variations of GFP that fluoresce with other colours are now available, allowing more than one label to be used simultaneously (Fig. 1.5). Photographs of GFP-labelled embryo and cells are courtesy of Tom Pratt, University of Edinburgh, UK; photograph of GFP-labelled adult mouse reprinted from Hadjantonakis, A-K., Gertsenstein, M., Ikawa, M., Okabe, M. and Nagy, A. (1998) Generating green fluorescent mice by germline transmission of green fluorescent ES cells. *Mechanisms of Development* **76**, 79–90 with permission from Elsevier.

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genes discovered initially in *Drosophila* could be targeted and shown to play critical roles in 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.\(^{12}\)

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

Fig. 1.7 Reverse genetics: generation of transgenic mice. This method allows the experimenter to manipulate specific genes in a mammalian species so as to learn about their functions. For example, a normal gene might be replaced with a non-functional version to generate a knock-out. To do this, the genome of embryonic stem cells (see Fig. 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 × symbols), carrying the central portion into the genome to prevent the function of the target gene. (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.
mechanisms of development of neural structures found in humans. 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 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?

1.5.5 Humans

We cannot experiment on humans, nor can we manipulate human embryos during nervous system development. However, it is important to recognize that major contributions to developmental neurobiology have been made by identifying genes whose disruption in humans is associated with disease. Studies in human genetics have now been complemented by the sequencing of the entire human genome. The identification of numerous genes that are critical for normal developmental processes has been achieved by analysing the genome of patients with genetic disease. Examples will be given in later chapters. How this type of research is done is outside the scope of this book.13 In overview, however, this approach can be seen as an excellent example of forward genetics, working from phenotype to gene.

1.5.6 Other vertebrates

Other mammalian species that have proved useful for studying specific aspects of development, for example cerebral cortical development, include the rat, guinea pig, hamster, ferret, cat and monkey. None have the advantages of the mouse for molecular genetics but they do have 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

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13 We suggest Part 3 of Human Molecular Genetics by T. Strachan and A. P. Read (Garland Science, 2004) for a detailed account.
developing nervous system at an earlier stage. Cats 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 recognized by the award of a Nobel Prize to David Hubel and Torsten Wiesel in 1981\(^{14}\) (see also Box 9.5 in Chapter 9). Monkeys have the advantage of being closely related to humans and they have been used particularly to study the development of neural connections and function. Work using these species will be discussed mainly in the book’s final chapters (especially Chapters 9 and 12).

### 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. 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 9.

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 manipulating the functions of genes 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 its expression to discover its functions.
- A major breakthrough in understanding mammalian neural development was the application of transgenic methods for manipulating the genome of mice.
- Some areas of developmental neurobiology are amenable to the application of computational modelling approaches to test hypotheses theoretically prior to the use of experimental methods.