Stem cells — hype and hope

Ron McKay

Studies of stem cells will help in understanding the development and function of organs in mammals. They may also offer a way of treating diseases ranging from liver failure to Parkinson's disease.

Politicians, journalists, patients… it seems as though everyone is talking about stem cells. Why all the fuss? Stem cells have become the centre of so much attention because they turn into all the different cell types that make up complex organisms, and they promise to perform this remarkable feat on demand. The implications for medicine are profound, but practical and ethical barriers stand in the way. Are our hopes for this technology justified?

Germ cells and cancer

All cells come from other cells, so what does it mean to be a stem cell? There are complex academic definitions, but the most important idea is that stem cells are undifferentiated — unspecialized — cells that can renew themselves and also give rise to one or more specialized cell types with specific functions in the body. This concept applies to many situations in biology and medicine. For example, tumours start off as just a single cell and, in many tumours, several cell types are derived from the tumour 'stem' cell. An extreme example of this phenomenon is seen in tumours of the testis called teratomas or teratocarcinomas. In mice, these tumours can be generated simply by taking normal testis cells and placing them at a new site. The observation that single tumour cells can generate many different cell types has had dramatic consequences.

Stem cells are defined by their developmental potential. The idea of a stem cell has a natural meaning in the context of the germ line; after all, the germ line of animals produces eggs or sperm, which together generate whole organisms. The fertilized egg, or zygote, is a 'totipotent' (from the Latin *totus*, meaning 'entire') stem cell, and generates all the cells of an animal — including those that do not form part of the embryo, such as the cells of the placenta. As development proceeds, cells become channelled into particular pathways of differentiation, and their developmental potential becomes modified. Stem cells in these pathways can differentiate easily into a limited number of cell types; for example, stem cells in the brain ultimately give rise to all the different types of neuronal and non-neuronal cells in the central nervous system.

It would seem reasonable to expect the existence of stem cells during development, but they also occur in adult tissues. For example, adult muscle stem cells can rebuild a muscle, and haematopoietic stem cells in adults can restore all the different cell types found in blood.

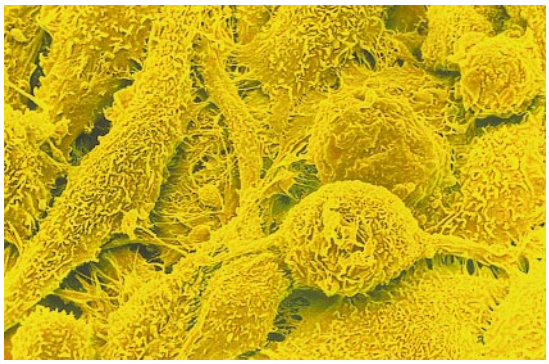
Embryonic stem cells

Inspired by the work on teratomas, scientists soon realized that there is a restricted period during early mouse development when certain normal cells have a remarkable ability to differentiate into a huge variety of cell types. These early embryonic stem (ES) cells can be taken from the embryo and grown in the laboratory; when placed back into a developing embryo, they contribute to all of the tissues of the mouse — including the germ line[1]. Such cells are said to be 'pluripotent' (*plures*, meaning several). The difference between the pluripotent ES cell and the totipotent zygote is that the ES cell can only generate cells that form part of the embryo itself. Another type of pluripotent cell is the embryonic germ (EG) cell. These come from a later stage in development, when the cells of the germ line become set aside.

The ES and EG cells also exist in humans, and it has become possible over the past few years to grow them in the lab[2–4]. The mouse ES cell can be easily defined by its ability to contribute to all the tissues of a developing embryo, as normal mice can be derived from ES cells. But it is illegal worldwide to derive a person from human ES cells, so the experimental definition of such a cell must be couched in different terms. Human ES cells are generally obtained from the extra embryos that are generated in fertility clinics. At a very early stage, when the embryo consists of only about a thousand cells, they can be separated from each other and grown in tissue culture. They can give rise to many different cell types in culture, so this is how they are defined — as pluripotent cells in tissue culture.

There is clear academic interest in culturing mouse ES cells and placing them back in developing embryos (Box 1). But what is the motivation for working with early human cells? Broadly speaking, there are two answers. First, these cells can be used to investigate features that are specific to early human development. Second, ES cells generate somatic cell types — the variety of non-reproductive cells that make up the human body. By studying ES cells, we may gain a deeper understanding of the process

Box 1 Learning about gene function



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Mouse embryonic stem (ES) cells, shown here, can be grown in large numbers for long periods in the laboratory, and their genes can be altered in precise ways while they are being cultured. The effects of these genetic changes can then be assessed by making the cells differentiate into other cells, either in a culture dish or after placing the cells into the blastocyst — an early stage of development in mammals. The ES cells become incorporated into the blastocyst and then become a part of the developing mouse. The importance of this technique cannot be exaggerated. Defining the functions of the thousands of mammalian genes is a central goal of modern biomedical research, and mouse ES cells will help us to achieve it.

Precise genetic manipulation is not restricted to ES cells. In a study published last month[23], genes in sheep fibroblast cells were altered by genetic recombination. The nuclei of the fibroblast cells were then transferred into eggs from which the nuclei had been removed, and the eggs were allowed to develop into sheep containing the desired genetic change. The benefits of genetically modifying farm animals in this way might include the ability to generate strains of cattle free of the protein agent underlying bovine spongiform encephalopathy (BSE). **R.M.**

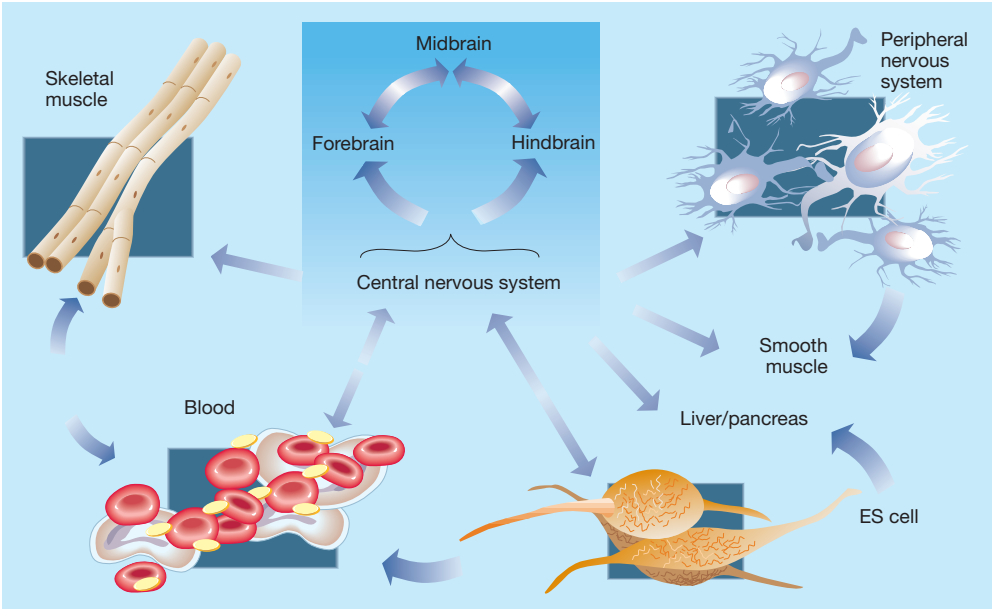

Figure 1 **Stem-cell transitions. At least in the lab, stem cells are not always restricted to one particular pathway of differentiation. For example, central nervous system (CNS) stem cells form the different cell types of the CNS, but can also differentiate into haematopoietic (blood) stem cells. Blood stem cells in turn form the different cell types found in blood, as shown here, but can also differentiate into skeletal muscle stem cells (which differentiate into skeletal muscle cells, pictured) and central nervous system cells. Embryonic stem (ES) cells are pluripotent, and contribute to all of the tissues of developing mammals. For simplicity, only a few of the stem-cell types that ES cells can produce are shown here.**

of cell replacement. The potential benefits to human health are huge, and range from generating new neurons for treating patients with Parkinson's disease to learning about the molecular processes that drive the development of tumours.

But the origins of human ES cells pose obvious ethical obstacles to their use in research (Box 2). One way round this problem might be provided by the observation that, during adulthood, the cells in most tissues are replaced — so there may be a source of new cells in the adult.

Adult stem cells

From the time that Henri Becquerel and Marie Curie discovered radiation, it has become increasingly clear that radiation damages rapidly dividing cells. It causes disease most readily in the white and red cells of the blood (the haematopoietic system). These observations led eventually to the identification of the haematopoietic stem cell (HSC). An experimental definition of the HSC is that, amazingly, just one cell can reconstitute the entire blood system of a mouse[5].

The fact that adult stem cells exist is exciting enough, but even more intriguing is that the potential of stem cells does not seem to be restricted by their source (Fig. 1). For example, a series of startling observations indicates that muscle and blood might be obtained from stem cells found in the tissues of either system[6,7]. With all the genetic markers and protocols at our disposal for identifying HSCs, it will not take long to test this proposition — that exactly the same stem cells can generate the entire blood system as well as the striated muscle that allows you to run for the bus.

Stem cells are abundant in the developing brain and in two areas of the adult central nervous system (CNS): the hippocampus and the olfactory bulb. Cells in the brain are not replaced as efficiently as blood cells, however, so we cannot define adult CNS stem cells in the same way as haematopoietic stem cells. But CNS stem cells can be easily grown in the lab and — under the right conditions — differentiate efficiently in culture dishes into neurons, oligodendrocytes (the cells that insulate the electrical signals passing down axons in the nervous system) and astrocytes (another type of non-neuronal cell in the CNS). The many cell types of the peripheral nervous system are also generated from a stem cell.

Mouse CNS stem cells can also differentiate to form the cells of other organs, including muscle, blood, intestine, liver and heart[8,9].

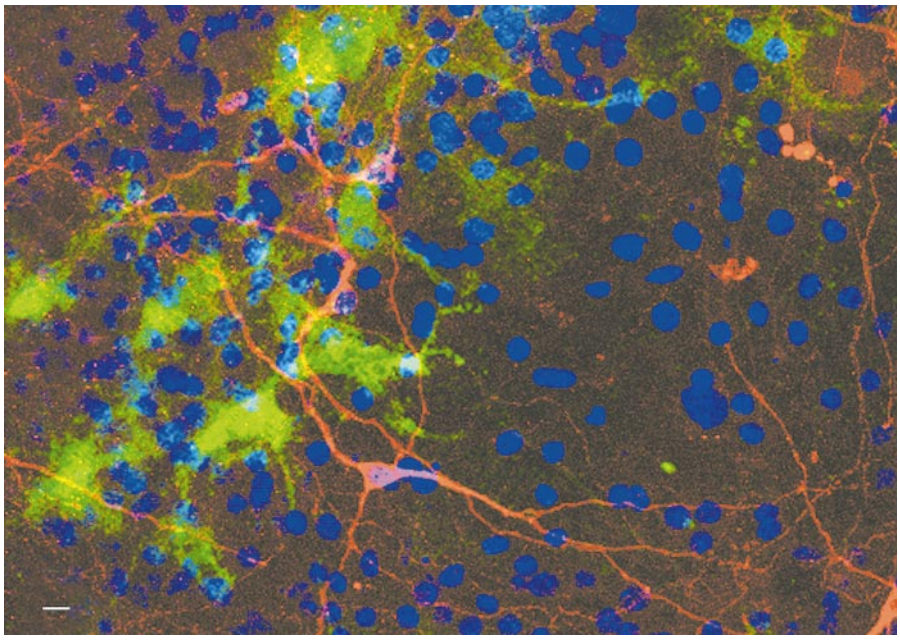

Figure 2 **Dopamine-producing neurons derived from mouse embryonic stem (ES) cells[15]. ES cells (labelled with green fluorescent protein) were induced to differentiate into midbrain neurons that make the enzyme tyrosine hydroxylase (labelled with a red dye). These neurons make dopamine and form synapses with their normal brain targets when cultured together with those targets. Nuclei are labelled with a blue dye. The ability to generate dopamine-producing neurons from ES cells may provide an unlimited source of these neurons for clinical work and drug discovery. Scale bar, 20 μm.**

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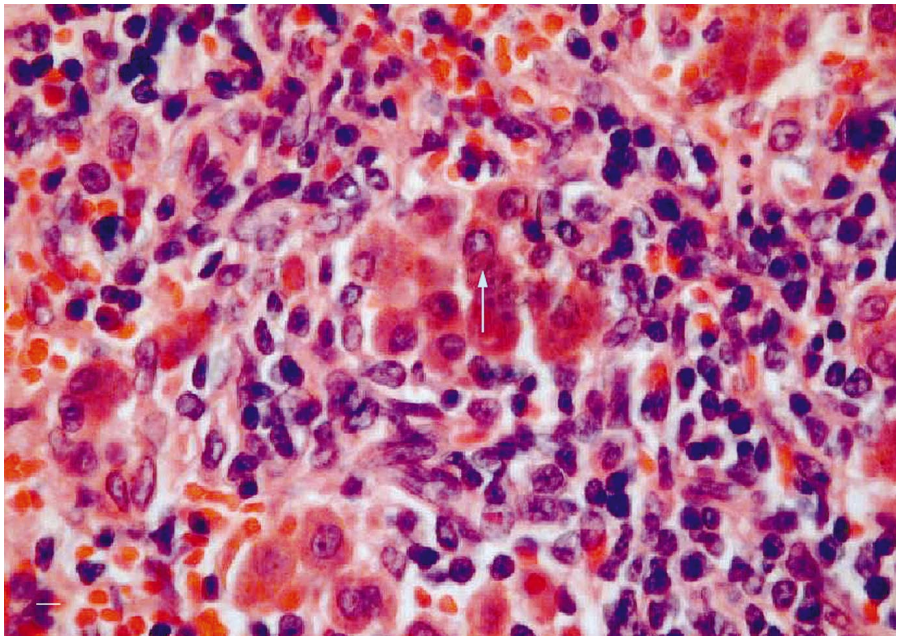

Figure 3 **In principle, liver diseases might be treatable by transplanting isolated liver cells (hepatocytes). Donor livers, however, provide only a fraction of the cells needed for clinical transplantation, and primary liver cells do not proliferate significantly in tissue culture. When hepatocyte stem cells have been immortalized by introducing growth-promoting genes into them, they proliferate in culture, retain their ability to differentiate into hepatocytes, and function in animal models of liver failure[16]. This photomicrograph of a spleen section shows a transplanted cell (arrow) with hepatocellular morphology three months after transplantation. Scale bar, ~10 μm.**

Conversely, blood cells can differentiate into brain cells[10]. It seems that adult stem cells may have the same developmental potential as ES cells, if given the right cues. But we know little about how stem cells can differentiate across boundaries, and how we could divert them into the pathway of choice. And — so far — no adult human stem cells have been shown to have such potential.

Mammals appear to contain some 20 major types of somatic stem cell. For example, stem cells have been described that can generate liver, pancreas, bone and cartilage. So it may be possible to obtain a wide range of stem cells from sources other than ES cells. This solution would effectively sidestep the ethical problem of using ES cells. But we have much to learn about how stem cells replicate and differentiate, and ES cells may offer essential technical advantages over other sources of somatic precursors.

Clinical potential and pitfalls

One reason for studying stem cells is their usefulness in cell-replacement therapy. The manipulation of HSCs is already an important clinical tool, and human HSCs have been essential in bone-marrow grafts that are in wide clinical use, for example in treating leukaemia patients. Although we are not certain of the identity of the stem cell concerned, skin cells can also be grown in large numbers, providing a life-saving grafting treatment for burn victims. And diabetes might be treatable by generating from stem cells the insulin-producing cells found in the normal pancreas and grafting them into the pancreas of diabetic patients.

Cell-replacement therapy also has potential in treating brain disease[11]. When grafted into the developing or adult mouse brain, CNS stem cells can differentiate into neurons and glial cells (non-neuronal cells in the nervous system) that are incorporated into the host tissues. Such grafted stem cells might be able to correct brain diseases characterized by loss of neurons. This idea is best developed for Parkinson's disease: there are encouraging findings showing that stem cells from the fetal brain could be used to restore some brain function to Parkinson's patients[12].

But we need routine access to the right cells to make cell grafting a practical technology. For example, the neurons required to treat Parkinson's disease can be obtained from the fetal brain, but there are technical and ethical barriers to using this tissue source. We can now generate the relevant neurons — those that produce the neurotransmitter dopamine — from mouse CNS stem cells, which can multiply and differentiate in the lab[13]. But the CNS stem cells quickly lose their ability to differentiate into dopamine-producing neurons. For this and many other cell types, it may not be possible to grow many stem cells in large enough numbers for cell-replacement strategies.

The pluripotent ES cell has an important advantage over somatic stem cells: it can be grown indefinitely in tissue culture. So it may be easier to obtain some of the cells that we need (such as dopamine-producing neurons for treatment of Parkinson's disease; Fig. 2) by culturing ES cells and prompting them to differentiate into the correct cell type. As yet, little is known about human ES cells, but many different cell types can be obtained from mouse ES cells in culture. Given the right combination of cues such as growth factors, these ES cells can differentiate *in vitro* into oligodendrocytes[14] — the cells that are missing in patients with multiple sclerosis — and into the neurons that die during Parkinson's disease[15]. In humans, oligodendrocytes can probably be obtained from sources less controversial than ES cells. But at the moment the only source of unlimited numbers of the neurons appropriate for Parkinson's patients is ES cells.

There may be other ways of obtaining enough adult stem cells for treatment purposes. While studying cancer, researchers have identified many genes involved in controlling cell growth. 'Immortalized' cells grow indefinitely in culture, without forming tumours when injected into animals. Many stem-cell types can be immortalized by introducing growth-promoting genes into them; after immortalization, they retain the ability to differentiate. Strikingly, immortalized cells can incorporate into the host tissue when they are grafted into animals. For example, earlier this year researchers showed that immortalized human liver cells, grafted into rats with acute liver failure (Fig. 3), can keep such rats alive[16]. There is a risk with using immortalized cells — they may be more likely to develop into tumour cells. A way around this problem is exemplified by the work with immortalized liver cells, which were generated with a 'cell-suicide' gene that could be activated by administering a drug to the rats. Strategies of this kind could allow cell numbers to be controlled and immortalized cells to become a clinically useful technology.

All in all, cell replacement is taking a central place in medicine, and the work discussed here is just a beginning. We do not know where unexpected benefits may suddenly emerge. For example, individual stem cells move easily through tissue and might be used to track down and kill cancer cells[17]. Another outcome of research into stem cells might be an understanding of how to direct the stem cells already present in our bodies to the necessary cell fates, without the need to

Box 2 Knowledge, ethics and laws

François Raspail understood as early as 1825 that cells are always derived from other cells ("*Omnis cellula e cellula*"; see ref. 24), that cells are not created equal, and that the mechanisms that generate differences between cells would explain the organization of cellular order in living organisms. These ideas provide the basis for modern studies of stem-cell biology. Knowledge gives us power, and the decision to act, or not to act, on the basis of that knowledge[25]. A greater understanding of stem cells brings the power to treat diseases and we must decide how best to go about this.

Doubts arise mainly from the fact that many useful stem cells are derived from embryos or fetuses. Although in most cases the embryos would otherwise be discarded, the question remains as to whether the extraction of embryonic stem (ES) cells from such embryos is justified. On the other side of the coin, this technology has great potential for the treatment of disease. There are alternatives: adult mouse stem cells (for example, muscle stem cells) can be diverted into different cell lineages. But their developmental potential might not be as great as that of ES cells, and it is difficult to extract and culture them.

Legislation around the world at present reflects this quandary. For example:

- In Germany, laws on reproductive medicine ban the extraction of stem cells from a human embryo.
- In the United States, federal funds cannot be used to conduct research using human pluripotent stem cells obtained from human fetal tissue or human embryos. The main federal funding agency, the National Institutes of Health, is investigating its guidelines on this issue at present. A bill recently discussed by a Senate subcommittee proposed legislation that would allow researchers to obtain human ES cell lines with federal funding. At the time of writing, this bill had not yet been voted on. Private funding of research using ES cells is not prohibited in the United States.
- In Britain, it is illegal to derive ES cells from embryos or fetuses. But it is legal for UK researchers to import ES cells from abroad. The government is still debating whether or not to allow researchers to derive ES cells in the United Kingdom.
- French bioethics laws do not allow research on human embryos, but a recent report, adopted by the assembly general of the Conseil d'Etat, recommended that embryo research should be allowed for the purposes of research into stem cells. This issue is to be debated later this year. **R.M.**

isolate and culture them. Such manipulation of resident stem cells may be clinically and ethically better than techniques in which cells are grown in the lab and grafted into the body where needed.

This may not be science fiction. In the brain, most neurons are generated during development, but, in the olfactory bulb and the hippocampus, neurons are still formed in the adult. The hippocampus is important in forming new memories, and, given recent success in restoring cell proliferation in this brain region in aged rats[18], it might be possible to develop pharmacological methods to regulate the formation of new neurons in the hippocampus and to minimize memory loss during ageing. We might be able to apply this approach to many neurological problems, as replacement of neurons in the brain by the differentiation of intrinsic stem cells may be much more widespread than we previously thought[19]. But before we can recruit these resident stem cells, we need to know much more about the mechanisms that control their birth, fate and death.

Controlling cell fate

We are used to thinking of animal design as a consequence of the actions of an inexorable developmental machine. First there is the totipotent egg and then, through a series of irreversible restrictions in developmental potential, the differentiated cell types of the animal are obtained. From this perspective, cells in adult tissues are seen as having few options. But, as discussed above, even adult animals contain stem cells with extraordinary developmental plasticity. This implies that the key events of development still occur in the 70-year-old heart or the 95-year-old brain.

The behaviour of stem cells depends on their history and on their local context or niche. They are not inflexible; we must simply learn how to influence them. Single extracellular signals that interact with cell-surface signal-detecting proteins can direct brain and blood stem cells to specific fates[20–22]. For example, immature B cells (white blood cells) can be propagated for long periods in the presence of a cytokine called interleukin-7 (ref. 22). In the absence of interleukin-7 they differentiate into mature B cells. If a key gene transcription factor, Pax5, is missing from the immature B cells, they can be nudged into a variety of different cell types, such as dendritic cells (antigen-presenting cells) or macrophages (which engulf foreign material), depending on the combination of cytokines added to the culture medium. Isolating the fewer than 20 somatic stem-cell types, and defining their responses to external signals, will help us to decipher the language used to build and maintain tissues. We may also learn how to guide stem cells along desired pathways of differentiation, both *in vitro* and *in vivo*.

Conclusion

The wide distribution of stem cells raises some fundamental questions. Why are animals built this way? What other models of multicellularity can we imagine? Are there similar stem cells in model experimental organisms such as the fruitfly *Drosophila melanogaster* and the nematode worm *Caenorhabditis elegans*? These questions are interesting in their own right, and stem-cell biology will lead to a better understanding of the way in which animals work. But the history of modern biology is that even apparently obscure scientific work can bring practical benefit. Perhaps the answers to these fundamental questions will bring progress in transforming advances in stem-cell biology into a reduction in the burden of disease. When it comes to using human stem cells, particularly ES cells, there are clear ethical dilemmas (Box 2). But it is my belief that there is also a moral imperative in moving discoveries in this field to the clinic. ■

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1. Brook, F. A. & Gardner, R. L. *Proc. Natl Acad. Sci. USA* **94,** 5709–5712 (1997).
2. Thomson, J. A. *et al. Science* **282,** 1145–1147 (1998).
3. Shamblott, M. J. *et al. Proc. Natl Acad. Sci. USA* **95,** 13726–13731 (1998).
4. Reubinoff, B. E., Pera, M. F., Fong, C.-Y., Trounson, A. & Bongso, A. *Nature Biotechnol.* **18,** 399–404 (2000).
5. Osawa, M., Hanada, K., Hamada, H. & Nakauchi, H. *Science* **273,** 242–245 (1996).
6. Jackson, K. A., Mi, T. & Goodell, M. A. *Proc. Natl Acad. Sci. USA* **96,** 14482–14486 (1999).
7. Gussoni, E. *et al. Nature* **401,** 390–394 (1999).
8. Bjornson, C., Rietze, R., Reynolds, B., Magli, M. & Vescovi, A. *Science* **283,** 534–537 (1999).
9. Clarke, D. L. *Science* **288,** 1660–1663 (2000).
10. Eglitis, M. A. & Mezey, E. *Proc. Natl Acad. Sci. USA* **94,** 4080–4085 (1997).
11. Snyder, E., Taylor, R. & Wolfe, J. *Nature* **374,** 367–370 (1995).
12. Olanow, C. W., Kordower, J. H. & Freeman, T. B. *Trends Neurosci.* **19,** 102–109 (1996).
13. Studer, L., Tabar, V. & McKay, R. D. *Nature Neurosci.* **1,** 290–295 (1998).
14. Brüstle, O. *et al. Science* **285,** 754–756 (1999).
15. Lee, S.-H., Lumelsky, N., Studer, L., Auerbach, J. M. & McKay, R. D. *Nature Biotechnol.* **18,** 675–679 (2000).
16. Kobayashi, N. *et al. Science* **287,** 1258–1262 (2000).
17. Benedetti, S. *et al. Nature Med.* **6,** 447–450 (2000).
18. Cameron, H. A. & McKay, R. D. *Nature Neurosci.* **2,** 894–897 (1999).
19. Magavi, S. S., Leavitt, B. R. & Macklis, J. D. *Nature* **405,** 951–955 (2000).
20. Anderson, D. J. *Trends Genet.* **13,** 276–280 (1997).
21. McKay, R. *Science* **276,** 66–71 (1997).
22. Cory, S. *Nature* **401,** 538–539 (1999).
23. McCreath, K. J. *et al. Nature* **405,** 1066–1069 (2000).
24. Harris, H. *The Birth of the Cell* (Yale Univ. Press, New Haven, CT, 1999).
25. Monod, J. *Chance and Necessity* (Knopf, New York, 1971).

Related websites

- **http://www.aaas.org/spp/dspp/sfrl/projects/stem/main.htm**
- **http://bioethics.gov/pubs.html#stemcell**
- **http://www.nih.gov/news/stemcell/index.htm**
- **http://www.nuffield.org/bioethics/**