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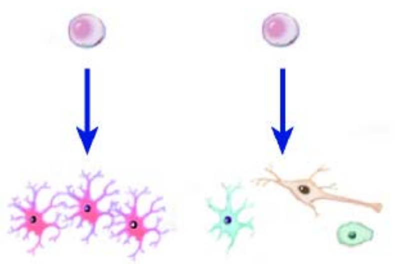
What are stem cells?

Stem cells – their origin and capacity, with emphasis on neuronal stem cells

During the development of an embryo, all organs are created from groups of proliferating (dividing) cells, stem cells, which have the capacity to give rise to all different cell types in a whole, functioning organism. The term stem cell describes a cell that (I) has a prolonged or unlimited capacity for self-renewal, and (II) has multi-potency, *i.e.*, can differentiate (develop) into cells of multiple lineages. (Fig 1).



Figur 1. Unipotent cells can produce but one cell type; multipotent cells can produce several cell types.

A few days after fertilization the mammalian egg is in the blastocyst stage and is implanted into the uterus. Some of the cells in the blastocyst develop into outer layer and inside this layer there is a mass of cells. The outer layer develops into membranes of the foetus (amnion, chorion) and placenta, while the inner cell-mass develops into the embryo.

A totipotent stem cell from a fertilized egg can give rise to a full organism, including the entire central and peripheral nervous system as well as the placenta. A pluripotent stem cell is restricted in that it can give rise to every cell of the organism, including cells of the nervous system, except the outer cell layer of the embryo at the blastocyst stage. Embryonic stem cells (ES cells) are derived from the cells forming the inner cell mass in the blastocyst and belong to the pluripotent class of cells (Fig. 2).

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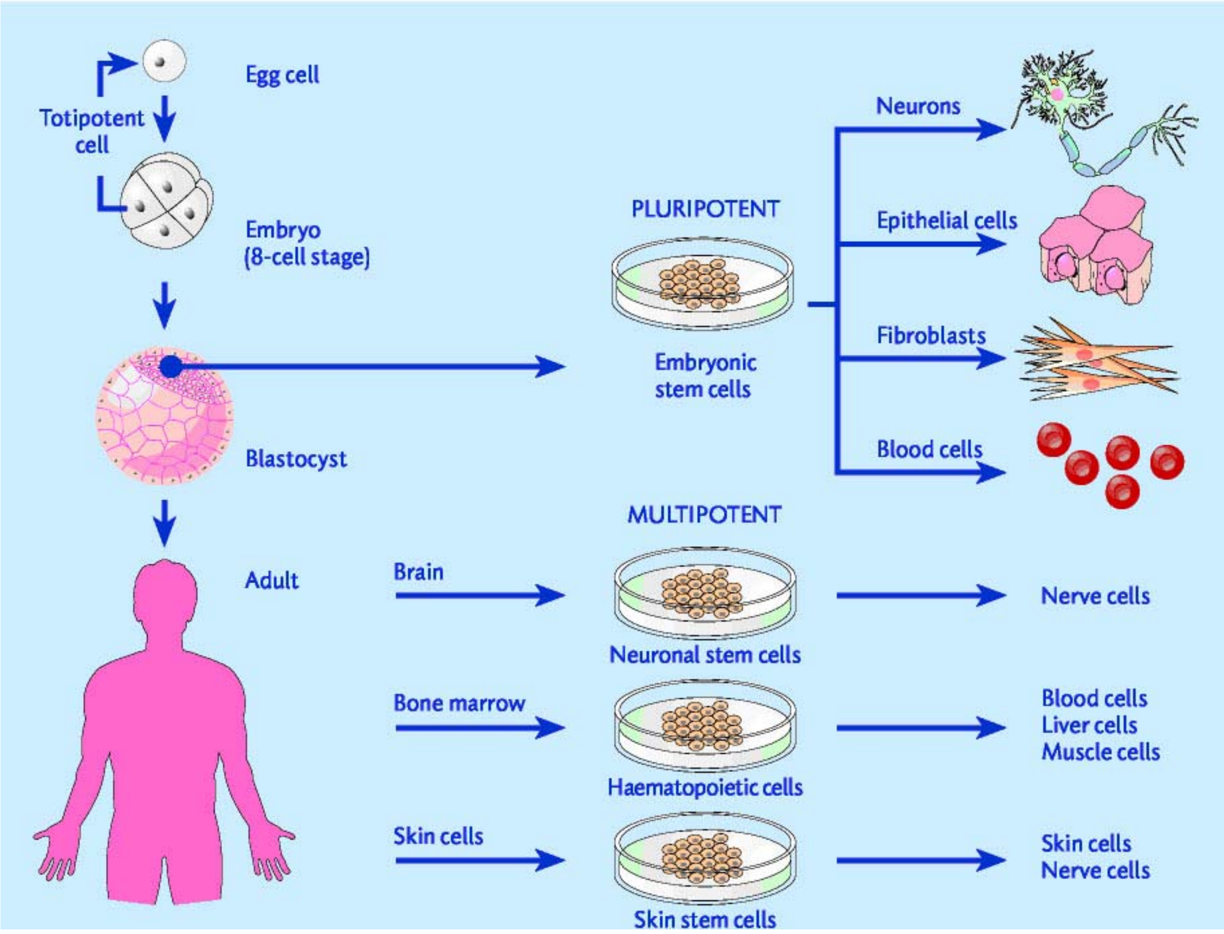

Figure 3. Stages of differential progression when a stem cell turns into a fully differentiated cell (Source: European Commission, Research DG-© European Communities, 1995-2002)..

Some stem cells remain undifferentiated and maintain their ability of self-renewal even in the adult organism. These stem cells are referred to as adult stem cells. They are found in various tissues such as the liver, the retina, bone marrow and intestine where they lie dormant until needed, for example to replace cells lost through injury or for tissue maintenance. Adult stem cells belong to the multipotent stem cells. The developmental potential of the multipotent cells has not yet been fully tested. Until recently it was believed that these cells only could give rise to the same kind of cells as from the organ where they originally were derived, but several studies have challenged this theory. Some stem cells from the adult brain have for example been proposed to be able to give rise to most cell types when injected into mouse or chick early embryo. Similarly, cells from adult bone marrow have been proposed to become neurons under the right conditions though they originally have a commitment to give rise to blood cells. It has been debated whether there is a universal adult stem cell, as potent as the embryonic stem cell. Reports of skin tissue giving rise to, *e.g.*, neural cells and neural stem cells contributing to muscle cells or blood cells can give the impression that there indeed exists such a cell. However, to date no population of adult stem cells capable of forming all kinds of cells has been isolated..

Progenitor cells descend from stem cells but are more limited concerning proliferation and differentiation. While an adult stem cell is self-renewing and multipotent, a progenitor cell has a limited capacity for self-renewal and is more likely to differentiate into a specific cell type. When a stem cell divides at least one of the two descendents usually is a stem cell with the capability to replicate itself, but when a progenitor cell divides it can form two specialized cells that are not capable of replicating themselves. Therefore, proliferation in a culture of progenitor ceases over time. Neuronal progenitor cells give rise to neurons or glia (astrocytes and oligodendrocytes), the three main cell types in the brain (Fig 3a, b and c).

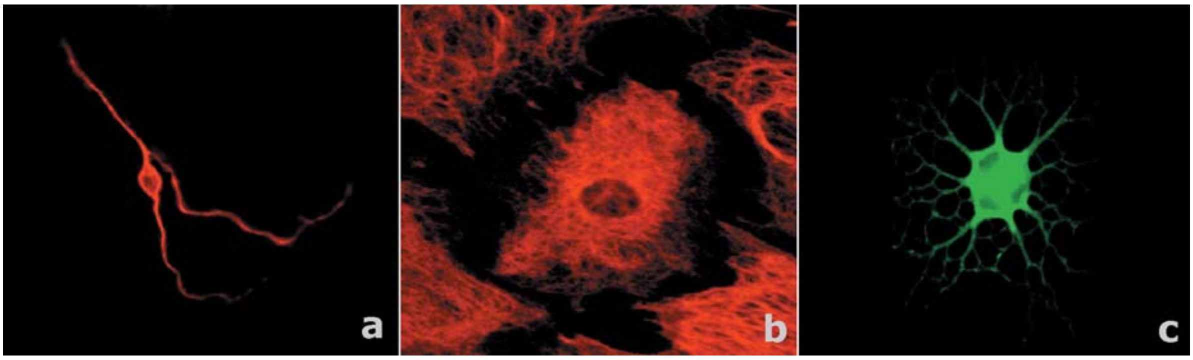

Figure 3. The three main cell types in the brain viewed through a fluorescence microscope; a) a neuron, b) an astrocyte and c) an oligodendrocyte.

Discovering proliferation in the adult brain

Although the presence of stem cells in various tissues has been known for more than a century, the adult brain has long been thought to be an exception due to poor regenerative capacity. Throughout the 20th century, neural regeneration was described in terms of an attempt of the nervous system to keep neurons alive after injury and to promote axonal growth by stimulating the neuronal environment. The generation of new neurons (neurogenesis) was believed only to occur during the development of an embryo and perinatally, except of the formation of new synapses in the adult brain. However, recently it was shown that stem cells do exist in the adult central nervous system (CNS), including the adult human brain. This was quite unexpected. The first studies with a positive outcome focused on the hippocampus (see next section) and the olfactory bulb of the adult rodent brain, where proliferation of new neurons was demonstrated. Further studies of rodents and songbirds as well as fish, frogs and reptiles pointed in the same direction: that neurons could be formed after birth. Since then numerous studies have been conducted and today all species studied, including humans, have shown this capacity of extensive cell division, self-renewal and neurogenesis. It is an important discovery as this potentially can be used for therapeutic purposes in brain areas damaged by trauma or diseases such as Parkinson's, multiple sclerosis and Alzheimer's (Fig.4).

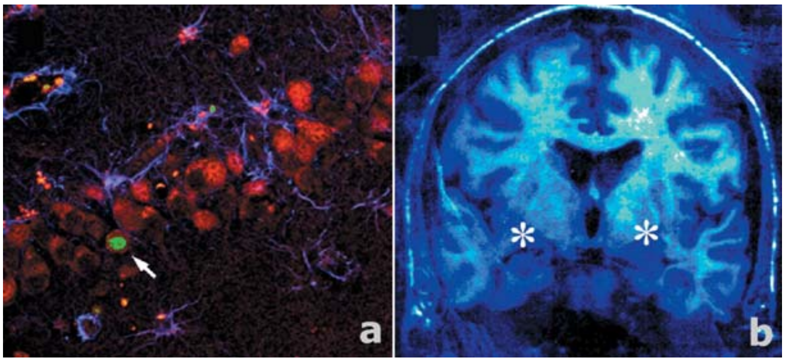

Figure 4. a) Bromodeoxyridine (BrdU) is incorporated in the DNA of dividing cells and is used to label newly born neurons, here in the hippocampus of an adult human (arrow).
b) Magnetic resonance imaging (MRI), a brain imaging technique, showing a cross-section of the human brain. The asterisks show the location of the hippocampus (Eriksson et al., 1998).

Neurogenesis in the adult rat hippocampus

The structure called the *hippocampus* (from the Greek word for "seahorse") is a piece of folded cortex on each side of the lateral ventricles (Fig. 5). It has been shown in rats that an hippocampal area called the dentate gyrus (DG) contains neural stem cells that postnatally can differentiate into neurons, astrocytes and oligodendrocytes (Fig.6). Since the hippocampus is involved in memory and learning, plasticity (capacity for change) here is of great interest when the ongoing renewal of neurons can be a way of adapting to changing environment and stimuli.

Another area that has shown to host cells with stemcell properties is the subventricular zone (SVZ) of the lateral ventricles. SVZ is probably the main source of adult stem cells (Fig.6).

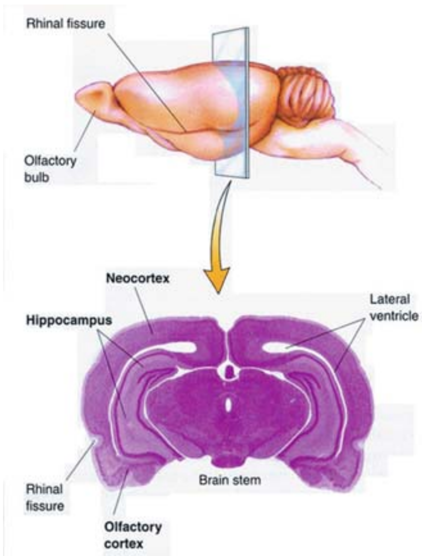

Figure 5. A section of a rat brain, showing the hippocampus and the lateral ventricles *From*: Bear, et al. © Lippincott Williams and Wilkins, 2001.

Factors influencing adult neurogenesis and cell survival

New methods for studying the molecular mechanisms underlying the regulation of neurogenesis have provided us with a range of possible factors involved in the production and survival of late-generated neurons and glia. The knowledge of the mechanisms is still fragmentary, but it appears to be the environment, rather than the intrinsic properties of the cells themselves, that dictates their fate. When grafted to the developing brain, fetus-derived stem cells migrate along with the host cells and differentiate into cell types specific for the target region. This is also true for stem cells derived from adult tissue. To get enough of them they can be cloned in a laboratory and when treated with growth factors and implanted back they generate new neurons and glia. Although it is known that hormones, neurotransmittors and growth factors are involved in the regulation of neurogenesis, the exact molecular and cellular signals leading to a certain choice of pathway are still to be revealed. Better knowledge of the mechanisms controlling proliferation and differentiation is necessary. Adult stem cells are rare and difficult to isolate, and their proliferation in culture is limited. The use of stem cells to replace degenerating tissue in neurological disease or injured tissue after trauma is of great interest, yet not enough is known about the properties of the cells and there is also a need to develop more effective methods to rapidly generate large numbers of human adult stem cells for transplantations.

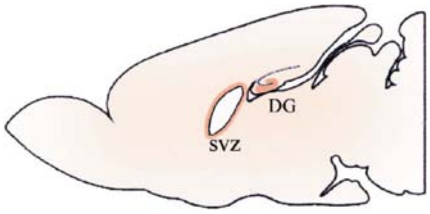

Figure 6. Areas known to generate new neurons in the adult CNS are the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ).

Exercise enhances neurogenes

There is nothing new about the fact that exercise makes us healthier. What´s new is that this also seems to be true for our neural stem cells. Mice given the opportunity to exercise in a running wheel show increased neurogenesis and numbers of surviving newborn cells in the dentate gyrus. Running rats show an increased resistance to brain insult such as stroke as well as improved recovery. A study conducted on humans in 2001 further proved the importance of physical activity, revealing lower risks of brain diseases such as dementia and Alzheimer's. Moreover, voluntary exercise is thought to be an excellent way to relieve stress. Stress inhibits the production of new neurons in the adult dentate gyrus, has a negative impact on neuronal survival and also impairs spatial memory.

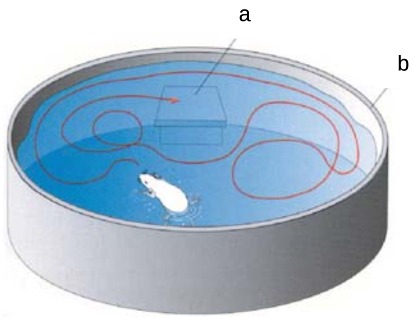

Figure 7. A Morris' water labyrinth. a) shows the hidden platform and b) the swimming-pool. *From*: Bear, et al. © Lippincott Williams and Wilkins, 2001.

The hippocampus shows high synaptic plasticity and is often used as a model for signal transmission. Synaptic plasticity in the hippocampus is thought to contribute to the involvement of the hippocampus in memory and learning. The importance of the hippocampus in memory and learning is shown in a so-called Morris water maze, a test of spatial memory in rats (Fig. 7). The rat is placed in a pool filled with milky water where a platform is submerged just beneath the surface. Normally, a naive rat will swim until it bumps into the platform, and it will soon learn to swim straight to it. But a rat with a hippocampal damage does not seem to remember the location of the platform, no matter how many trials. Running enhances neurogenesis in the dentate gyrus and memory performance in the water maze task, a sign of improved brain plasticity.

Embryonic stem cells

An embryonic stem (ES) cell is, as already mentioned, an undifferentiated pluripotent cell derived from the early embryonic stage, called a blastocyst (see Fig.2). The ES cells have the unique ability to proliferate indefinitely in vitro without differentiating and thus maintaining the potential to differentiate into derivates of all three embryonic germ (EG) layers. The EG layers which comprise the ectoderm (skin and nerve cells), the mesoderm (e.g. muscle cells, connective tissues and erythrocytes) and the endoderm (internal organs) are the source of all cell types in the adult body (around 250 different cell types). However, it is important to stress that embryonic stem cells are not themselves able to give rise to an embryo, as they lack the capability to form the cells that build up the outer cell layer of the blastocyst, which later form the placenta and other supportive tissues necessary for development in the uterus.

Isolation of a human embryonic stem cell line

In Sweden spare (cleavage-stage) human embryos, produced by in vitro fertilization (IVF) are used to isolate human embryonic stem cell lines. Pairs participating in the IVF program can after informed consent donate extra embryos. Pluripotential embryonic stem cells have been derived very efficiently from these human embryos and grown in culture. The inner cell mass (ICM) is isolated from a day-5 embryo (by immunosurgery or enzymatic dissolving) and plated onto a feeder cell layer in tissue cultures. The feeder cells are derived from mice and produce substances that induce cell growth. When the ICM is removed from its normal embryonic environment and cultured under appropriate conditions, it gives rise to cells that show the hallmarks of stem cells and express a number of markers for embryonic stem cells.

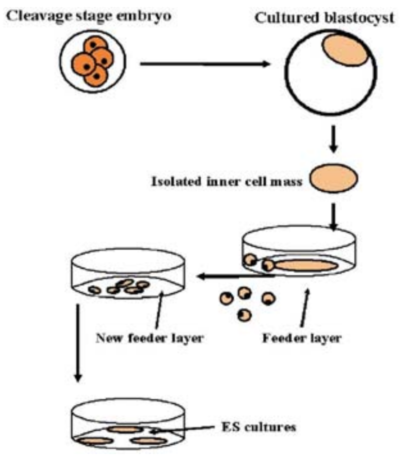

Figure 8. The process for generating a pluripotent embryonic stem cell line from a blastocyst.

The ICM outgrowths propagate in the presence of serum and after 9-15 days they have divided and formed clumps; cells from the periphery of these clumps are chemically or mechanically dissociated and replated in identical culture conditions. Colonies with the appropriate undifferentiated morphology are subsequently selected, removed, dissociated and replated. The cells are then expanded and passaged, thus creating a potential embryonic stem cell line (Fig. 8).

Stem cells as a therapeutic use: Why use embryonic stem cells??

The ethics around using human embryonic stem cells for therapies and research is a well-debated area today. Why not only use the adult stem cells for these purposes and avoid the difficult ethical questions like "when does life begin"?

There are several reasons for doing research with human embryonic stem cells. One is that ES cells have an unlimited capacity to proliferate in vitro, which has not yet been shown for adult stem cells. Furthermore, ES cells probably can generate a more diverse repertoire of cell types than adult stem cells.

There is also a problem generating enough cells for therapies within a limited time. Adult stem cells exist in small numbers decreasing with age and they are difficult to isolate in pure form, whereas ES cells can be grown in large cultures and expanded for an indefinite time. To use autologous adult stem cells (the patient's own stem cells), the cells must first be isolated from the patient and then cultured until there is enough for therapeutic use. This is a time-consuming process, which may be a problem if treating an acute disease. More importantly, extensive time in vitro leading to a number of (doublings) cell divisions may induce genetic alterations.

Another shortcoming of adult stem cells concerns the treatment of genetic diseases. Autologous adult stem cells might not be suitable for transplantation since they also carry the defective gene. Moreover, adult stem cells may contain several DNA defects acquired through exploitation to every day life, like sunlight, toxins, and normal DNA replicating errors, defects that would be avoided using ES cells.

Research conducted on embryonic stem cells can also help us to understand fundamental events in early human development such as identifying important genes and the factors regulating their activation and inactivation, as well as understanding chromosomal defects and errors causing abnormal cell differentiation and division, leading to cancer and a wide range of other diseases.

Stem cell therapy in neurological disorders

There is a big potential in using stem cells to alleviate a wide range of diseases. When it comes to transplantation, stem cells are a very promising field of research. It is difficult to find immunologically matching donors, and a donation from family members is required in order to obtain organs and tissues for transplantation. Stem cells could be used to produce tissues such as bone, cartilage and liver cells and in the future even whole organs for transplantation. Moreover, stem cells can be genetically modified to replace defective genes before transplantation back into the patient and this could be of potentially important clinical use in, as an example, the case of severe autoimmune diseases.

The pathological lesions of many neurological diseases are often widely disseminated in the brain and spinal cord and therefore, transplantation has until now not been an option as a therapeutic strategy. Although the adult mammalian brain contains endogenous (its own) neuronal stem cells, self-repair of lesions caused by trauma or neurological diseases is often poor. This could be due to an insufficient number of endogenous stem cells as well as an unfavorable microenvironment. It is likely that stem cells are present throughout the whole CNS, but the differentiation into neurons seems to be restricted to a few areas in vivo, predominantly the olfactory bulb and the dentate gyrus. The capability of transplanted neuronal stem cells to migrate and integrate host tissue and additionally express therapeutic molecules, has given us a new possibility to treat neurological diseases. Degeneration of specific neuronal cell types can be a result of toxins, insufficiency of certain trophins or other pathologic processes. The ideal graft would not only repair damage but also produce therapeutic molecules, stabilizing the microenvironment and act protecting against further damage. It has already been shown that neuronal stem cells can produce a wide range of trophic factors. Neuronal stem cells are also easy to administer; they can spread directly from the cerebral ventricles and possibly also pass the blood-brain barrier. It has been demonstrated that neuronal stem cells implanted at birth into the brain of a (the meander tail (mea)) mutant mouse, which has a deficiency in granule cell neurons, will "choose" to become granule cells; shifting their differentiation to compensate the deficiency in granule cells.

In the case of multiple sclerosis, the demyelination of axons can cause severe dysfunction in humans. Myelin, provided by oligodendrocytes, normally acts as an insulating sheet around the axons of the nerve cells, facilitating the conduction of nerve impulses. In a mouse model mimicking the demyelination in multiple sclerosis, transplanted neuronal stem cells differentiate towards oligodendroglia and remyelinate a large number of the injured host axons, giving a decrease of symptoms. Successful stem cell therapy in humans with Parkinson's disease has already begun using transplanted fetal cells that produce dopamine.

The examples presented above are currently the most promising studies conducted on neuronal stem. However, there are numerous of serious obstacles to overcome before effective stem cell based therapies can be widely applied. As already mentioned, endogenous factors creating an environment unsuitable for transplantation may hamper therapeutic use. Moreover, the high rate of proliferation among stem cells could lead to problems such as ageing and cancer, and the long-term effects on the characteristics of manipulated stem cells is still not known. Further experiments mapping the different factors that, in a positive or negative way, regulate and affect the survival, proliferation and differentiation of neuronal stem cells and progenitors are therefore necessary.

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