

Stem Cells in Medicine

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Why are stem cells so fascinating? Besides their capacity for unlimited lifespan, one feature that makes stem cells desirable to medicine is a unique ability to repopulate and potentially restore defective tissues and organs. Following injury, cells in the body migrate to replace and fill up the damaged area. Albeit the human body cannot regrow a lost arm or leg. Given that there are animals whose limbs can regrow, such capabilities have likely been lost during evolution. There are two main types of stem cells: embryonic and adult stem cells.



Embryonic Stem Cells

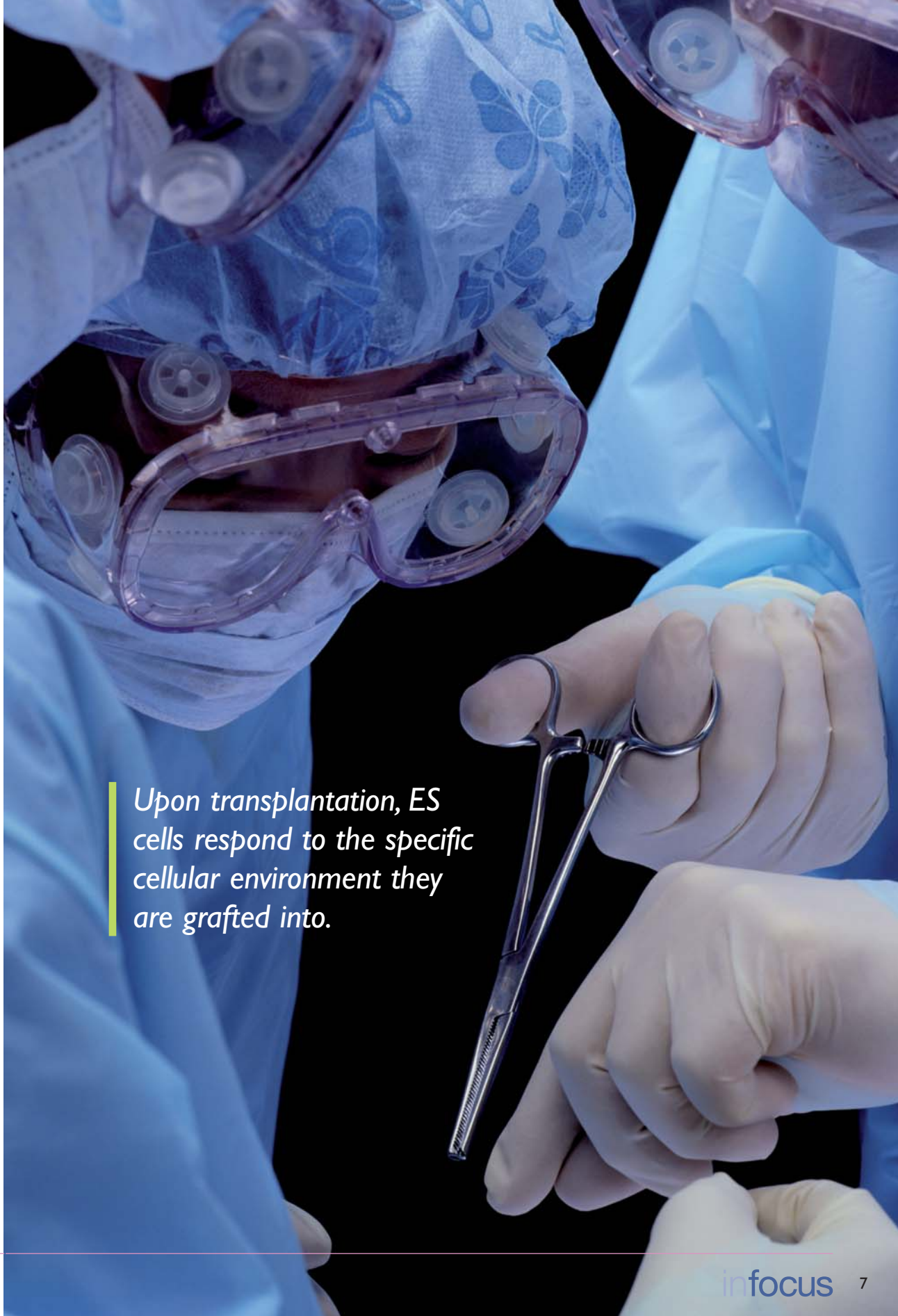
Embryonic stem (ES) cells are cell lines maintained in the laboratory, which have originally been derived from very early embryos. ES cells are retrieved from the so called inner cell mass of blastocysts, which forms after a few divisions of the fertilized egg. Consequently, ES cells are retrieved from cultured in vitro fertilized eggs before implantation into the uterus. Remarkably, when ES cells are transplanted into a blastula, an early stage of embryonic development in animals, they can turn into any cell type in the whole body. The ability for differentiation into all cell types in the body is reproduced in culture, where ES cells have been shown to produce numerous well-defined cell types and even tissues. Moreover, ES cells are said to be pluripotent since they can give rise to all three germ layers: ectoderm; mesoderm; and endoderm. Human embryonic stem cells potentially make up a renewable source of more differentiated cells, which may be used for cellular transplantation to replace damaged or diseased tissue in patients. However, already in 1970 it was observed that early mouse embryos grafted into adult mice produced teratocarcinomas. Teratocarcinomas are malignant germ cell tumours containing undifferentiated embryonal carcinoma cells, which can be propagated in culture. Similarly, when injected into adult mice ES cells form teratomas, which are tumours with tissue or organ components resembling normal derivatives of all three germ layers.

So how can stem cell induced tumours be avoided? One possible route may be pre-differentiation of ES cells into the desired cell type before transplantation. Even so, a problem that remains to be solved is the inevitable presence of some undifferentiated ES cells among the population of pre-differentiated cells to be transplanted. Thus before pre-differentiated ES cells can be used as treatment in a clinical setting, it would be essential

to develop methodology for complete elimination of ES cells which have escaped differentiation.

Upon transplantation, ES cells respond to the specific cellular environment they are grafted into. It has been demonstrated that neuronal differentiation occurs when ES cells are introduced into a developing mouse brain. In adults, transplantation of ES cell derived glial cells can generate re-myelination in a rat model for multiple sclerosis, and partial functional recovery has been obtained in a spinal cord injury model. How far down the differentiation pathway cells need to have gone before transplantation remains an open question, and most likely depends on the type and location of the transplanted tissue. Both external regulatory signals and internal cellular responses, like epigenetic modifications of chromatin and the formation of metastable transcription factor networks, are required to maintain stem cell properties and to suppress differentiation.

ES cells are routinely cultured on a feeder layer, which consists of mitotically inactivated mouse embryonic fibroblast cells. One extrinsic factor, a single cytokine leukemia inhibitory factor (LIF), has been found to sustain unlimited lifespan and self renewal of mouse ES cells in the absence of feeder layers, and vice versa LIF withdrawal induces mouse ES cell differentiation (Smith, 2001). LIF activate the transcription factor STAT3 through the gp130 receptor. Both LIF and the gp130 receptor are expressed in the early embryo. Though experiments indicate that they are not required for development prior to gastrulation, the early development phase of animal embryos during which the morphology of the embryo is dramatically re-structured by cell migration. In contrast to mouse ES cells, human ES cells appear independent of LIF and may utilize alternative pathways to sustain unlimited lifespan and self-renewal. It has been shown that oocytes,



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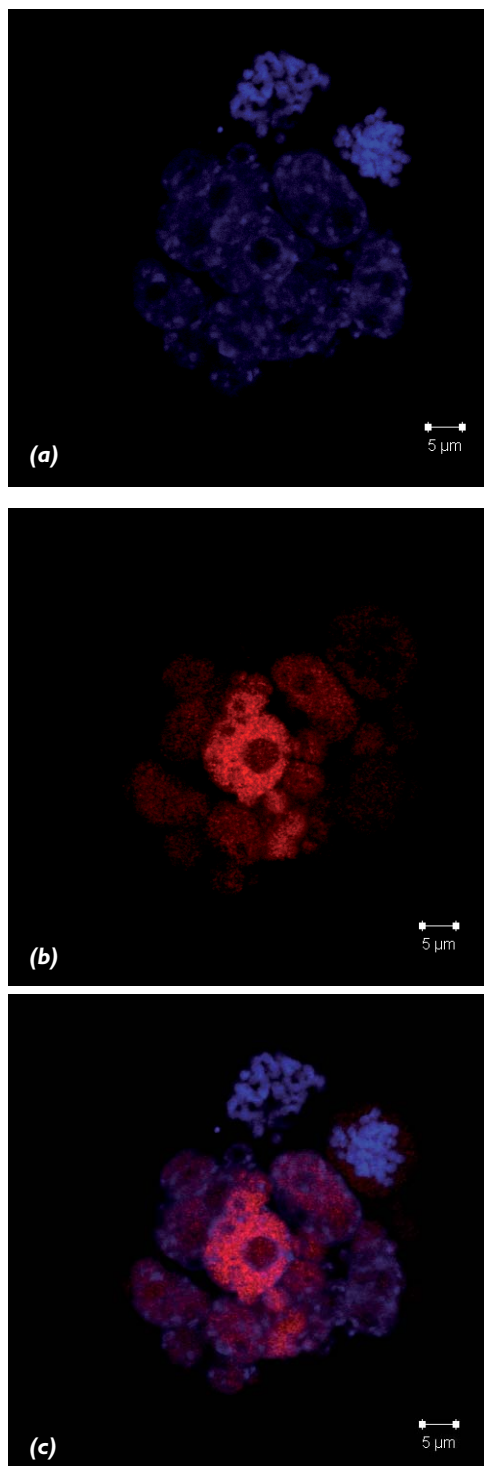


Fig. 1. Immunolocalisation of Oct4 in ES cells. Confocal images of an embryonic stem cell niche. Scale bars represent 5 μm. (a) DAPI staining of DNA (blue), (b) Oct4 specific antibody (red), (c) Merge of DAPI and Oct4 staining.

female germ cells involved in reproduction, from amphibians can bring adult mammalian nuclei back to a stem cell state (Byrne *et al.*, 2003). This suggests that factors involved in nuclear reprogramming events are also required for stem cell maintenance. The stem cell specific protein Oct4, which is a marker for pluripotency, appears to be of particular importance. The Oct4 protein is critical for early development, for nuclear transfer experiments to succeed, and for maintenance of ES cells. It is a transcription factor that controls the activity of several other genes that are important during development. Successful nuclear transfer experiments do not merely depend on the presence of Oct4, but also requires precise Oct4 dosage. Confocal microscopy (Figure 1) reveals that Oct4 is down-regulated upon differentiation of embryonic stem cells. A central stem cell contains very high levels of the Oct4 protein, whereas the surrounding cells only contain moderate Oct4 levels. Two peripheral cells have been arrested in cell division chemically and exhibit highly condensed chromosomes. Both cells have started to differentiate and only contain minute amounts of Oct4 protein.

Therapeutic Cloning

Transplantation of ES cells, as with organs, can invoke an immune response that may cause rejection. One way around immune rejection is to create ES cells from somatic cells, such as skin cells. This is referred to as somatic nuclear transfer, or therapeutic cloning. From the earliest somatic nuclear transfer experiments in amphibians (Gurdon *et al.*, 1958) to the creation of mammals like the sheep Dolly (Wilmut *et al.*, 1997), it has become clear that somatic nuclei successfully transplanted into eggs undergo major reprogramming of gene expression. Therapeutic cloning thus appears to depend on the reversal of epigenetic marks that give somatic cells their

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characteristic features. Therapeutic cloning techniques involve transplantation of a cell nucleus into an egg, where factors yet unknown reprogram the nucleus to lose its somatic cell characteristics, and to assume the characteristics of an embryonic stem cell. Before transplantation of the nucleus it is necessary to remove or destroy the egg’s own genetic material (DNA). A new clone, which is a copy of the donor, begins to form when the egg starts to divide. Classical ways to trick the egg to divide are exposure to calcium or subjecting it to an electrical force field. More research is required to optimise and mimic signals that normally initiate embryonic development.

After just a few cell divisions ES cell primary cultures can be derived from the inner cell mass of the preimplantation blastocyst stage of the embryo. Even though the success rate is very low for creation of a new individual by nuclear transfer the requirements to reprogram a nucleus in order to create a blastocyst are much more tolerable and have a higher success rate (Byrne *et al.*, 2002). Therefore, a lower threshold of reprogramming fidelity may be acceptable to derive useful human ES cells for medical purposes.

Adult Stem Cells

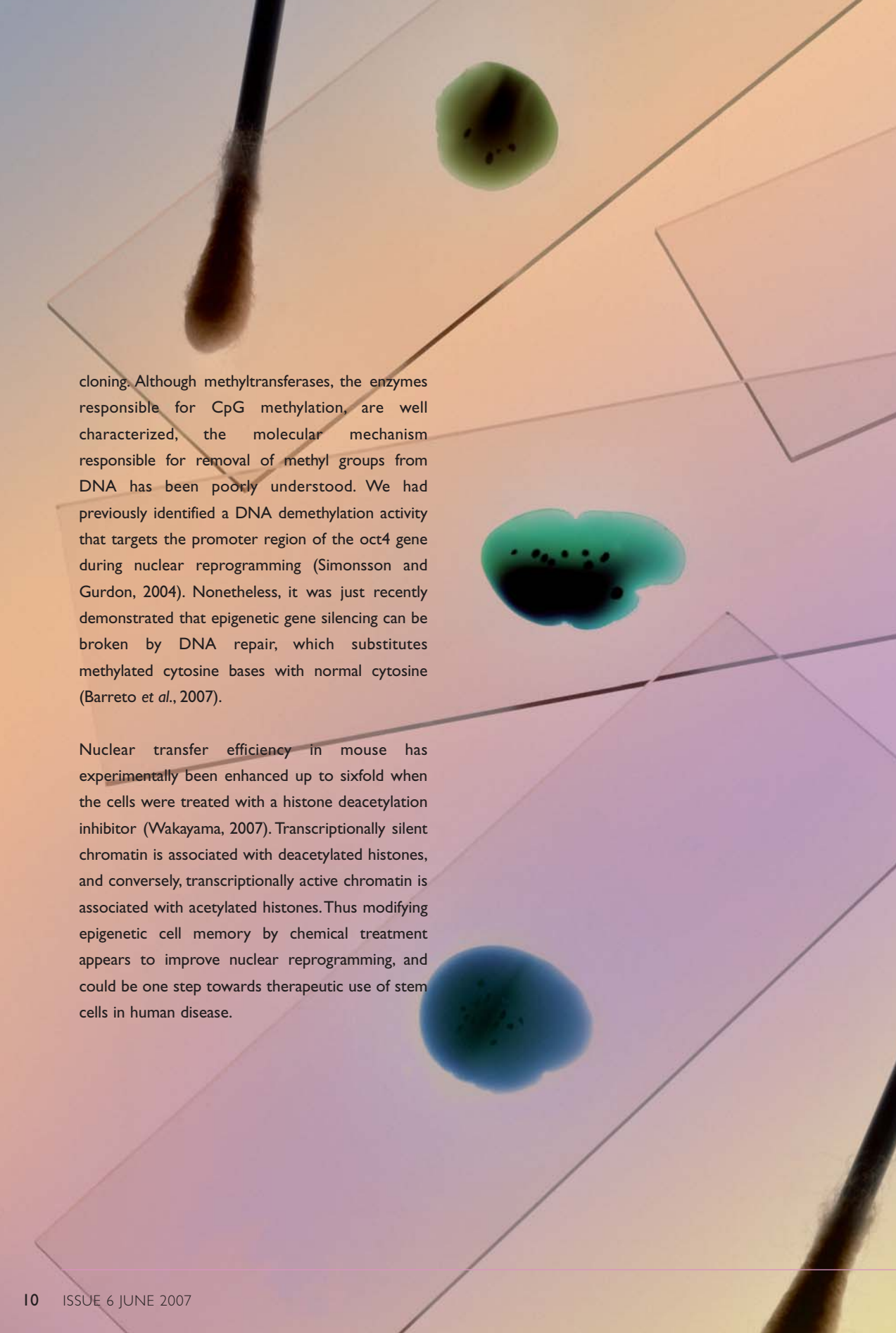
As development proceeds, the embryo forms two types of adult stem cells, germ line stem cells for reproduction and somatic stem cells for organogenesis. The cellular microenvironment surrounding both types of adult stem cells, and signals emanating from nearby differentiated supporting cells, is referred to as the niche (Li and Xie, 2005). In an organism, adult stem cells support ongoing tissue regeneration, replacing cells lost due

to injury or natural cell death by apoptosis. To sustain these functions throughout life, a sensitive balance between self-renewal and differentiation must be maintained. The underlying mechanisms that control this sensitive balance are fundamental to understand stem cell regulation, the nature of tumour formation and cancer diseases, and the therapeutic use of stem cells in human disease.

Epigenetic Cell Memory

As mentioned above, cells seem to have a capacity to ‘remember’ certain signals they have been exposed to. Molecular details of such cellular memory remain largely unknown. However, while the subject of genetics deals with inheritance through genes, whose DNA sequences encode information for cellular functions, the subject of epigenetics deals with reversible, yet heritable, changes in gene regulation that occur without changes in DNA sequences. Chromatin constituents like histone proteins are subject to methylation and acetylation to name but a few epigenetic marks. The collective epigenetic information of a somatic cell is generally thought to maintain a specific gene expression pattern, which is characteristic for that particular cell type. In addition, methylation of the DNA base cytosine, so called CpG methylation, can serve to permanently switch off the expression of embryonic genes. Control regions of housekeeping genes, which are expressed in all cell types, are devoid of CpG methylation. In contrast, the control regions of tissue specific genes are normally heavily CpG methylated in all tissues, except for the one where the gene is expressed.

Somatic epigenetic marks need to be erased for successful nuclear reprogramming in therapeutic



cloning. Although methyltransferases, the enzymes responsible for CpG methylation, are well characterized, the molecular mechanism responsible for removal of methyl groups from DNA has been poorly understood. We had previously identified a DNA demethylation activity that targets the promoter region of the oct4 gene during nuclear reprogramming (Simonsson and Gurdon, 2004). Nonetheless, it was just recently demonstrated that epigenetic gene silencing can be broken by DNA repair, which substitutes methylated cytosine bases with normal cytosine (Barreto et al., 2007).

Nuclear transfer efficiency in mouse has experimentally been enhanced up to sixfold when the cells were treated with a histone deacetylation inhibitor (Wakayama, 2007). Transcriptionally silent chromatin is associated with deacetylated histones, and conversely, transcriptionally active chromatin is associated with acetylated histones. Thus modifying epigenetic cell memory by chemical treatment appears to improve nuclear reprogramming, and could be one step towards therapeutic use of stem cells in human disease.

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Stina Simonsson received her PhD in 2000 at the Medical Biochemistry department of Gothenburg University, Sweden, where she studied initiation of DNA replication. She went on to become a BBSRC postdoctoral fellow in Professor Sir John Gurdon's lab in Cambridge, UK, between 2000 and 2005, where she explored nuclear reprogramming in cloned animals. Stina made the important discovery that DNA demethylation, which is an epigenetic marker of somatic mammalian cells, becomes erased in viable clones. She returned to Sweden in 2004, and currently leads a stem cell research group at Göteborg University, Institute of Biomedicine. Stina is currently funded by the Swedish research council, and she was awarded a female young researcher fellowship from the KAW Foundation in 2000, and was elected Åke Wiberg researcher of the year in 2006.



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Tomas Simonsson received his PhD in 1998 at the Biochemistry department of Chalmers University in Gothenburg, Sweden, where he made the discovery that the formation of a unique DNA structure is critical for the activation of cancer causing oncogenes. He was promoted to senior scientist in 2000, and obtained his postdoctoral training between 2000 and 2005 with Dr. Daniela Rhodes at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, where he started to explore how stem cells maintain unlimited lifespan. In 2006 he joined the Biomedicine department at the Sahlgrenska Academy of Gothenburg University, with a grant from the Swedish Research Foundation.



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Helena Johansson is a first year PhD student in Stina Simonsson's research group at Göteborg University, with a Degree of Master of Science in Pharmacy. Besides science she has a big interest in synchronized skating. She just recently won the World Synchronized Skating Championship in London, Canada, with Team Surprise, from Landvetter Sweden.