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1. Introduction

In 2006, Shinya Yamanaka and colleagues reported that only four transcription factors were needed to reprogram mouse fibroblasts back in development into cells similar to embryonic stem cells (ESCs). These reprogrammed cells were called induced pluripotent stem cells (iPSCs). The year after, iPSCs were successfully produced from human fibroblasts and in 2008 reprogramming cells were chosen as the breakthrough of the year by Science magazine. In particular, this was due to the establishment of patient-specific cell lines from patients with various diseases using the induced pluripotent stem cell (iPSC) technique. iPSCs can be patient specific and therefore may prove useful in several applications, such as; screens for potential drugs, regenerative medicine, models for specific human diseases and in models for patient specific diseases. When using iPSCs in academics, drug development, and industry, it is important to determine whether the derived cells faithfully capture biological processes and relevant disease phenotypes. This chapter provides a summary of cell types of human origin that have been transformed into iPSCs and of different iPSC procedures that exist. Furthermore we discuss advantages and disadvantages of procedures, potential medical applications and implications that may arise in the iPSC field.

1.1 Preface

For the last three decades investigation of embryonic stem (ES) cells has resulted in better understanding of the molecular mechanisms involved in the differentiation process of ES cells to somatic cells. Under specific in vitro culture conditions, ES cells can proliferate indefinitely and are able to differentiate into almost all tissue specific cell lineages, if the appropriate extrinsic and intrinsic stimuli are provided. These properties make ES cells an attractive source for cell replacement therapy in the treatment of neurodegenerative diseases, blood disorders and diabetes. Before proceeding to a clinical setting, some problems still need to be overcome, like tumour formation and immunological rejection of the transplanted cells. To avoid the latter problem, the generation of induced pluripotent stem (iPS) cells have exposed the possibility to create patient specific ES-like cells whose differentiated progeny could be used in an autologous manner. An adult differentiated cell has been considered very stable, this concept has however been proven wrong experimentally, during the past decades. One ultimate experimental proof has been cloning
Fig. 1. Schematic picture of establishment of patient-specific induced pluripotent stem cells (iPSCs), from which two prospective routes emerge: 1) in vivo transplantation, 2) in vitro human model system. Patient-specific induced pluripotent stem cells that are similar to embryonic stem cells (ESCs) are produced by first 1) collecting adult somatic cells from the patient, for example skin fibroblasts by a skin biopsy, 2) and reprogramming by retroviral transduction of defined transcription factors (Oct4, c-Myc, Klf4 and Sox 2 or other combinations) in those somatic fibroblast cells. Reprogrammed cells are selected by the detection of endogenous expression of a reprogramming marker, for example Oct4. 3) Generated patient-specific iPSCs can be genetically corrected of a known mutation that causes the disease. 4) Expansion of genetically corrected patient-specific iPSCs theoretically in eternity. First prospective route (Route 1): 5) upon external signals (or internal) iPSCs can theoretically be stimulated to differentiate into any cell type in the body. 6) In this way patient-specific dopamine producing nerve cells or skin cells can be generated and transplanted into individuals suffering from Parkinson’s disease or Melanoma respectively. Second route (Route 2): Generated disease-specific iPSCs can be used as a human in vitro system to study degenerative disorders or any disease, cause of disease, screening for drugs or recapitulate development.
animals using somatic cell nuclear transfer (SCNT) to eggs. Such experiments can result in a new individual from one differentiated somatic cell. The much more recent method to reprogram cells was the fascinating finding that mouse embryonic fibroblasts (MEFs) can be converted into induced pluripotent stem cells (iPSCs) by retroviral expression of four transcription factors: Oct4, c-My c, Sox2 and Klf4. iPSCs are a type of pluripotent stem cell derived from a differentiated somatic cell by overexpression of a set of proteins. Nowadays, several ways of generating iPSCs have been developed and includes 1) overexpression of different combinations of transcription factors most efficiently in combination with retroviruses (step 2 in Figure 1), 2) exposure to chemical compounds in combination with the transcription factors Oct4, Klf4 and retroviruses, 3) retroviruses alone, 4) recombinant proteins or 5) mRNA. The iPSCs are named pluripotent because of their ability to differentiate into all different differentiation pathways. Generation of patient-specific iPSC lines capable of giving rise to any desired cell type provides great opportunities to treat many disorders either as therapeutic treatment or discovery of patient specific medicines in human iPSC model systems (Figure 1). Here, some of this field’s fast progress and results mostly concerning human cells are summarized.

2. Reprogramming-Induced Pluripotent Stem Cells (iPSCs)

Reprogramming is the process by which induced pluripotent stem cells (iPSCs) are generated and is the conversion of adult differentiated somatic cells to an embryonic-like state. Takahashi and Yamanaka demonstrated that retrovirus-mediated delivery of Oct4, Sox2, c-Myc and Klf4 is capable of inducing pluripotency in mouse fibroblasts (Takahashi and Yamanaka, 2006) and one year later was reported the successful reprogramming of human somatic fibroblast cells into iPSCs using the same transcription factors (Takahashi et al., 2007). Takahashi and Yamanaka came up with those four reprogramming proteins after a search for regulators of pluripotency among 24 cherry picked pluripotency-associated genes. These initial mouse iPSC lines differed from ESCs in that they had a diverse global gene expression pattern compared to ESCs and failed to produce adult chimeric mice. Later iPSCs were shown to have the ability to form live chimeric mice and were transmitted through the germ line to offspring when using Oct4 or Nanog as selection marker for reprogramming instead of Fbx15, which was used in the initial experiments (Meissner et al., 2007; Okita et al., 2007; Wernig et al., 2007). Various combinations of the genes listed in table 1 have been used to obtain the induced pluripotent state in human somatic cells. The first human iPSC lines were successfully generated by Oct4 and Sox2 combined with either, Klf4 and c-Myc, as used earlier in the mouse model, or Nanog and Lin28 (Lowry et al., 2008; Nakagawa et al., 2008; Park et al., 2008b; Takahashi et al., 2007; Yu et al., 2007). Subsequent reports have demonstrated that Sox2 can be replaced by Sox1, Klf4 by Klf2 and c-Myc by N-myc or L-myc indicating that they are not fundamentally required for generation of iPSCs (Yamanaka, 2009). Oct4 has not yet been successfully replaced by another member of the Oct family to generate iPSCs which is logical due to the necessity of Oct4 in early development. However, BIX-01294 an inhibitor of G9a histone methyl transferase, which is involved in switching off Oct4 during differentiation, enables neural progenitor cells to be reprogrammed without exogenous Oct4, although transduction of Klf4, c-Myc and Sox2 together with endogenous Oct4 was required (Shi et al., 2008). Recently, Oct4 has been replaced with steroidogenic factor 1, which controls Oct4 expression in ESCs by binding the
Oct4 proximal promoter, and iPSCs were produced without exogenous Oct4 (Heng et al., 2010). Remarkably, exogenous expression of E-cadherin was reported to be able to replace the requirement for Oct4 during reprogramming in the mouse system (Redmer et al., 2011). iPSCs are similar to embryonic stem cells (ESCs) in morphology, proliferation and ability to form teratomas. In mice, pluripotency of iPSCs has been proven by tetraploid complementation (Zhao et al., 2009). Both ESCs and iPSCs can be used as the pluripotent starting cells for the generation of differentiated cells or tissues in regenerative medicine. However, the ethical dilemma associated with ESCs is avoided when using iPSCs since no embryos are destroyed when iPSCs are obtained. Moreover, iPSCs can be patient-specific and as such patient-specific drugs can be screened and in personalized regenerative medicine therapies immune rejection could be circumvented. However the question surrounding the potential immunogenicity remains unclear due to recent reports that iPSCs do not form teratomas probably because iPSCs are rejected by the immune system (Zhao et al., 2011).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct4</td>
<td>Transcription factor expressed in undifferentiated pluripotent embryonic stem cells and germ cells during normal development. Together with Nanog and Sox 2, is required for the maintenance of pluripotent potential.</td>
</tr>
<tr>
<td>Sox2</td>
<td>Transcription factor expressed in undifferentiated pluripotent embryonic stem cells and germ cells during development. Together with Oct4 and Nanog, is necessary for the maintenance of pluripotent potential.</td>
</tr>
<tr>
<td>Myc family</td>
<td>Proto-oncogenes, including c-Myc, first used for generation of human and mouse iPSCs.</td>
</tr>
<tr>
<td>Klf family</td>
<td>Zinc-finger-containing transcription factor Kruppel-like factor 4 (KLF4) was first used for generation of human and mouse iPSCs</td>
</tr>
<tr>
<td>Nanog</td>
<td>Homeodomain-containing transcription factor essential for maintenance of pluripotency and self-renewal in embryonic stem cells. Expression is controlled by a network of factors including the key pluripotency regulator Oct4.</td>
</tr>
<tr>
<td>Lin 28</td>
<td>Conserved RNA binding protein and stem cell marker. Inhibitor of microRNA processing in embryonic stem (ES) and carcinoma (EC) cells.</td>
</tr>
</tbody>
</table>

Table 1. Combinations of the genes that have been used to obtain the induced pluripotent state in human somatic cells

2.1 Differentiation of iPSCs into cells of the heart

After the cells have been reprogrammed, it will be possible to differentiate them towards a wide range of specialized cells, using existing protocols for differentiation of hESCs. Differentiation of beating heart cells, the cardiomyocytes, from hESCs has now been achievable through various protocols for a decade (Kehat et al., 2001; Mummery et al., 2002). In 2007, human iPScs were first reported to differentiate into cardiomyocytes (Takahashi et al., 2007), using a protocol including activin A and BMP4 which was described for differentiation of hESCs the same year (Laflamme et al., 2007). A comparison between the
cardiac differentiation potential of hESCs and iPSCs concluded that the difference between the two cell sources were no greater than the known differences between different hESC lines and that iPSCs thus should be a viable alternative as an autologous cell source (Zhang et al., 2009). Furthermore, a recent study demonstrated that reprogramming excluding c-MYC yielded iPSCs which efficiently up-regulated a cardiac gene expression pattern and showed spontaneous beating in contrast to iPSCs reprogrammed with four factors including c-MYC (Martinez-Fernandez et al., 2010). On the transcriptional level, beating clusters from both iPSCs and hESCs were found to be similarly enriched for cardiac genes, although a small difference in their global gene expression profile was noted (Gupta et al., 2010). Taken together, these results indicate that cardiomyocytes differentiated from both hESCs and iPSCs are highly similar, although differences exist.

2.2 Additional methods to achieve reprogramming - 1. cloning = Somatic Cell Nuclear Transfer (SCNT) 2. cell fusion 3. egg extract

In addition to the iPSC procedure other ways exist to reprogram somatic cells including: 1) somatic cell nuclear transfer (SCNT), 2) cell fusion of somatic adult cells with pluripotent ESCs to generate hybrid cells and 3) cell extract from ESCs or embryo carcinoma cells (ECs). From the time when successful SCNT experiments, more commonly known as cloning, in the frog *Xenopus Laevis* (Gurdon et al., 1958) to the creation of the sheep Dolly (Wilmut et al., 1997), it has been proven that an adult cell nucleus transplanted into an unfertilized egg can support development of a new individual, and researchers have focused on identifying the molecular mechanisms that take place during this remarkable process. Even though SCNT has been around for 50 years, the molecular mechanisms that take place inside the egg remain largely unknown. The gigantic egg cell receiving a tiny nucleus is extremely difficult to study. Single cell analysis are required and gene knock-out of egg proteins is very challenging. In 2007 a report that the first primate ESCs were isolated from SCNT blastula embryos of the species Rhesus Monkey was published (Byrne et al., 2007). The reason why it took so long to perform successful SCNT in Rhesus Monkey was a technical issue; to enucleate the egg, modified polarized light was used instead of traditional methods using either mechanical removal of DNA or UV light mediated DNA destruction. The first reliable publication of successful human SCNT reported generation of a single cloned blastocyst (Stojkovic et al., 2005). Unfortunately, the dramatic advances in human SCNT reported by Hwang and colleagues in South Korea were largely a product of fraud (Cho et al., 2006). In human SCNT reports, left over eggs from IVF (*in vitro* fertilization) that failed to fertilize have been used, indicating poor egg quality. However, human SCNT using 29 donated eggs (oocytes) of good quality, and not leftovers from IVF, from three young women were reported to develop into cloned blastocysts, at a frequency as high as 23% (French et al., 2008). Theoretically, hESC lines can be derived *in vitro* from SCNT generated blastocysts. However, so far no established hESC line using the SCNT procedure has been reported. The shortage of donated high quality human eggs for research is a significant impediment for this field.

Other methods that have been used to elucidate the molecular mechanism of reprogramming are 2) fusion of somatic adult cells with pluripotent ESCs to generate hybrid cells or 3) cell extract from ESCs or ECs (Bhutani et al., 2010; Cowan et al., 2005; Freberg et al., 2007; Taranger et al., 2005; Yamanaka and Blau, 2010).
3. Molecular mechanisms of reprogramming

The mechanisms of nuclear reprogramming are not yet completely understood. The crucial event during reprogramming is the activation of ES- and the silencing of differentiation markers, while the genetic code remains intact. Major reprogramming of gene expression takes place inside the egg and genes that have been silenced during embryo development are awakened. In contrast, genes that are expressed in, and are specific for, the donated cell nucleus become inactivated most of the time, however some SCNT embryos remember their heritage and fail to inactivate somatic-specific genes (Ng and Gurdon, 2008). It has been reported that reprogramming involves changes in chromatin structure and chromatin components (Jullien et al., 2010; Kikyo et al., 2000). Importantly, initiation of Oct4 expression has been found to be crucial for successful nuclear transfers (Boiani et al., 2002; Byrne et al., 2003) and important for iPSC creation; all other reprogramming iPSC transcription factors have been replaced with other factors or chemical compounds, but only one report so far could exclude Oct4. In murine ES cells, Oct4 must hold a precise level to maintain them as just ES cells (Niwa et al., 2000) and therefore understanding the control of the Oct4 level will be key if one wants to understand pluripotency and reprogramming at the molecular level.

A recent report demonstrated that Oct4 expression is regulated by scaffold attachment factor A (SAF-A). SAF-A was found on the Oct4 promoter only when the gene is actively transcribed in murine ESCs, depending on LIF, and gene silencing of SAF-A in ESCs resulted in down regulation of Oct4 (Vizlin-Hodzic et al., 2011). Other Oct4 modulators have been reported that in similarity with SAF-A are in complex with RNA polymerase II (Ding et al., 2009; Ponnusamy et al., 2009). Post-translational modifications have been shown to be able to modify the activity of Oct4, such as sumoylation (Wei et al., 2007) and ubiquitination (Xu et al., 2004). During the reprogramming process epigenetic marks are changed such as the removal of methyl groups on DNA (DNA demethylation) of the Oct4 promoter which has been shown during SCNT (Simonsson and Gurdon, 2004) and has also been observed in mouse (Yamazaki et al., 2006). The growth arrest and DNA damage inducible protein Gadd45a and deaminase Aid was shown to promote DNA demethylation of the Oct4 and Nanog promoters (Barreto et al., 2007; Bhutani et al., 2010). Consistent with those findings is that Aid together with Gadd45 and Mbd4 has been shown to promote DNA demethylation in zebrafish (Rai et al., 2008). Translational tumor protein (Tpt1) has been proposed to control Oct4 and shown to interact with nucleophosmin (Npm1) during mitosis of ESCs and such complexes are involved in cell proliferation (Johansson et al., 2010b; Koziol et al., 2007). Furthermore, phosphorylated nucleolin (Ncl-P) interacts with Oct4 during interphase in both murine and human ESCs (Johansson et al., 2010a). Core transcription factors, Oct4, Sox2 and Nanog, were shown to individually form complexes with nucleophosmin (Npm1) to control ESCs (Johansson and Simonsson, 2010). ESCs also display high levels of telomerase activity which maintain the length of the telomeres. The telomerase activity or Tert gene expression is rapidly down regulated during differentiation and are much lower or absent in somatic cells. Therefore, reestablishment of high telomerase activity (or reactivation of Tert gene) is important for reprogramming. In SCNT animals, telomere length in somatic cells has been reported to be comparable to that in normally fertilized animals (Betts et al., 2001; Lanza et al., 2000; Tian et al., 2000). A telomere length-resetting mechanism has been identified in the Xenopus egg (Vizlin-Hodzic et al., 2009).
When iPSCs first were introduced many thought that the molecular mechanism of reprogramming was solved once and for all. It was soon shown that to generate iPSC colonies one could use different combinations of transcription factors most efficiently together with retroviruses or more recently, exposure to chemical compounds together with the transcription factors, Oct4 and Klf4, and with retroviruses (Zhu et al., 2010) or retroviruses alone (Kane et al., 2010). What retroviruses do for the reprogramming process is unknown and the efficiency by which the egg reprograms the somatic cells is far more efficient than the iPSC procedure. Moreover, mutagenic effects have been documented in both laboratory and clinical gene therapy studies, principally as a result of a dysregulated host gene expression in the proximity of gene integration sites. So the first question to ask is whether all iPSC experiments so far forgot the obvious control of using only virus. The answer is probably no because the efficiency is very low with viruses alone as compared to using transcription factors combined with virus or identified reprogramming compounds. Reprogramming an adult somatic frog cell nucleus to generate a normal “clonal” new individual is far less efficient (0.1-3%) than reprogramming to create a blastocyst, from which ESCs are isolated (efficiency 20-40%) (Gurdon, 2008) and is comparable with blastula formation after human SCNT (23%). This number could be compared with iPSC procedure that has reported 0.5 % success rate at most with human cells (table 1). The low efficiency and slow kinetics of iPSC derivation suggest that there are other procedures that are more efficient, yet to decipher. There is a belief that there are different levels of pluripotency when it comes to ESC and also that reprogramming follows an organized sequence of events, beginning with downregulation of somatic markers and activation of pluripotency markers alkaline phosphatase, SSEA-4, and Fbxo15 before pluripotency endogenous genes such as Oct4, Nanog, Tra1-60 and Tra-1-80 become expressed and cells gain independence from exogenous transcription factor expression (Brambrink et al., 2008; Stadtfeld et al., 2008a). Only a small subset of somatic cells expressing the reprogramming factors down-regulates somatic markers and activates pluripotency genes (Wernig et al., 2008a).

3.1 History of reprogramming
SCNT has been around for more than fifty years although it was already proposed in 1938 by Hans Spemann (Spemann, 1938), an embryologist who received the Nobel Prize in Medicine for his development of new embryological micro surgery techniques. Spemann anticipated that “transplanting an older nucleus into an egg would be a fantastic experiment”. Later on, Robert Briggs and Thomas King were the first to put the nuclear transfer technique into practice. However, they only managed to obtain viable offspring through nuclear transfer of undifferentiated cells in the frog species Rana pipiens (Briggs and King, 1952). During the 1950s to the 1970s a series of pioneering somatic nuclear transfer experiments performed by John Gurdon showed that nuclei from differentiated amphibian cells, for example tadpole intestinal or adult skin cells could generate cloned tadpoles (Gurdon, 1962; Gurdon et al., 1958; Gurdon et al., 1975). In 1997, the successful cloning of a mammal was first achieved. The sheep Dolly was produced by using the nuclei of cells cultured from an adult mammary gland (Wilmut et al., 1997). Following the cloning of Dolly, researchers have reported successful cloning of a number of species including cow, pig, mouse, rabbit, cat (named Copycat) and monkey. In 2006, reprogrammed murine iPSCs were reported by Takahashi and Yamanaka (Takahashi and Yamanaka, 2006) and in 2007 human iPSCs were reported (Takahashi et al., 2007; Yu et al., 2009).
4. Producing iPSCs from other cell types than fibroblasts

The most studied somatic cell type that has been reprogrammed into iPSCs is fibroblasts. The different human somatic cell types that have been transformed into iPSCs so far are summarized in table 2. The efficiency of fibroblast reprogramming does not exceed 1-5% but generally is extremely inefficient (0.001-0.1%) and occurs at a slow speed (> 2 weeks). In order to use iPSCs in clinical applications, improved efficiency, suitable factor delivery techniques and identification of true reprogrammed cells are crucial. In the fast growing field of regenerative medicine, patient-specific iPSCs offer a unique source of autologous cells for clinical applications. Although promising, using somatic cells of an adult individual as starting material for reprogramming in this context has also raised concern. Acquired somatic mutations that have been accumulated during an individual’s life time will be transferred to the iPSCs, and there is a fear that these mutations may be associated with adverse events such as cancer development. As an alternative, iPSCs have been generated from human cord blood. These cells have been shown to differentiate into all three germ layers including spontaneous beating cardiomyocytes (Haase et al., 2009). Reprogrammed cells from cord blood have not only the advantage to come from a juvenescent cell source. In addition, cord blood is already routinely harvested for clinical use. Another issue that has been raised in this field is a wish to harvest cells for reprogramming without surgical intervention. Therefore, reprogramming experiments have also been performed using plucked human hair follicle keratinocytes. These iPSCs were also able to differentiate into cells from all three germ layers including cardiomyocytes (Novak et al., 2010).

<table>
<thead>
<tr>
<th>Human Origin Somatic Cell type</th>
<th>Efficiency</th>
<th>Reprogramming Factors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblasts</td>
<td>0.02%</td>
<td>OKSM</td>
<td>(Takahashi et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>0.02%</td>
<td>OSLN</td>
<td>(Yu et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>0.002%</td>
<td>OKS</td>
<td>(Nakagawa et al., 2008)</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>0.1%</td>
<td>OKSM</td>
<td>(Liu et al., 2010)</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>ND</td>
<td>OKSM</td>
<td>(Aasen et al., 2008)</td>
</tr>
<tr>
<td>Neuronal stem cells</td>
<td>&lt;0.004%</td>
<td>O</td>
<td>(Kim et al., 2008)</td>
</tr>
<tr>
<td>Amniotic cells</td>
<td>0.05-1.5%</td>
<td>OKSM</td>
<td>(Li et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>OSN</td>
<td>(Zhao et al., 2010)</td>
</tr>
<tr>
<td>Adipose-derived stem cells</td>
<td>0.5%</td>
<td>OKSM</td>
<td>(Sugii et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.1%</td>
<td>OKS</td>
<td>(Aoki et al., 2010)</td>
</tr>
<tr>
<td>Cord blood stem cells</td>
<td>ND</td>
<td>OKSM</td>
<td>(Eminli et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.01%</td>
<td>OS</td>
<td>(Giorgetti et al., 2009)</td>
</tr>
<tr>
<td>Cord blood endothelial cells</td>
<td>&lt;0.01%</td>
<td>OSLN</td>
<td>(Haase et al., 2009)</td>
</tr>
<tr>
<td>Mobilized peripheral blood</td>
<td>0.01%</td>
<td>OKSM</td>
<td>(Loh et al., 2009)</td>
</tr>
</tbody>
</table>

Table 2. Different somatic cell types that human iPSCs have been generated from

4.1 iPSC as a disease model

The introduction of iPSC technology holds a great promise for disease modelling. By differentiating iPSCs from patients into various cell lineages there is hope to be able to follow the disease progression and to identify new prognostic markers as well as to use the differentiated cells for drug screening in both toxicological testing and the development of
new treatment. This approach has already been tested for monogenic diseases using genetically modified hESCs or hESCs from embryos carrying these diseases (reviewed in (Stephenson et al., 2009)). However, diseases with a more complex genetic background involving several or unknown genes have not been able to be studied in this way before iPSCs became available. An additional advantage with iPSCs is that since many diseases differ in both clinical symptoms and penetrance between patients, iPSCs derived from patients will offer the opportunity to reveal a clinical history as well. It could also provide a model for late-onset degenerative diseases such as Alzheimer’s disease or osteoarthritis. Recent work on cardiac arrhythmias has fully shown the potential of disease modelling using iPSCs. Long QT syndrome (LQTS) is characterized by rapid irregular heart beats due to abnormal ion channel function and the condition can lead to sudden death. So far, various mutations in at least 12 different genes have been associated with LQTS and the disease is subdivided into different types depending on which gene is affected (reviewed in (Bokil et al., 2010)). Fibroblasts from patients with LQTS1 (Moretti et al., 2010) and LQTS2 (Itzhaki et al., 2011; Matsa et al., 2011) were reprogrammed and differentiated into the cardiac lineage. These cells displayed the electrophysiological pattern characteristic to the disease. Moreover, the cells responded appropriately when treated with pharmacological compounds, which further extends the usability of these cells.

iPSCs have also been generated from fibroblasts from patients suffering from the LEOPARD syndrome, an autosomal-dominant developmental disorder where one of the major disease phenotypes includes hyperthropic cardiomyopathy. The authors showed that cardiomyocytes derived from those iPSCs were larger with another intracellular organization compared to cardiomyocytes derived from hESCs or iPSCs generated from a healthy sibling (Carvajal-Vergara et al., 2010). Today many laboratories and hospitals worldwide are producing iPSC lines from patients with various diseases. Patient-specific iPSC lines can be used as 1) a human modelling system for studying the molecular cause of, and in the long run for 2) the treatment of, degenerative diseases with autologous transplantation, which refers to the transplantation to a patient of his/her own cells. The therapeutic potential of iPSCs in combination with genetic repair has already been successfully shown in mouse models of sickle cell anemia (Hanna et al., 2007), Duchenne muscular dystrophy (DMD) (Kazuki et al., 2010), hemophilia A (Xu et al., 2009) and, in a rat model, Parkinson’s disease (Wernig et al., 2008c). For diseases where animal and human physiology differ, disease-specific iPSC lines capable of differentiation into the tissue affected by the disease could recapitulate tissue formation and thereby enable determination of the cause of the disease and could provide cues to drug targets. Therefore iPSC lines from patients suffering from a variety of genetic diseases with either Mendelian or complex inheritance have been secured for future research, and include deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset (type1) diabetes mellitus (JDM), Downs syndrome (DS)/trisomy21 and Lesch-Nyhan syndrome (Park et al., 2008a). Furthermore, iPSCs derived from amyotrophic lateral sclerosis (ALS) patients were terminally differentiated into motor neurons (Dimos et al., 2008).

4.2 Procedures to produce iPSCs

In the first iPSC reprogramming studies, retroviral or lentiviral vectors were used to introduce the transcription factors into somatic cells. By using these viral delivery systems,
Fig. 2. Methods for producing induced pluripotent stem cells (iPSCs) by non-integrating vectors. Several different methods exist to generate iPSCs by non-integrating vectors:

- **a. Plasmids**: Transfect into somatic cells → Reprogramming → iPSC colony
- **b. Episomal vectors**: Transfect into somatic cells → Reprogramming → iPSC colony
- **c. Adenoviral vectors**: Infect into somatic cells → Reprogramming → iPSC colony
- **d. Minicircles vectors**: Transfect into somatic cells → Reprogramming → iPSC colony
- **e. mRNA**: Transfect into somatic cells → Reprogramming → iPSC colony
example by plasmid, episomal, adenoviral minicircle vectors and mRNA. a) A combination of expression plasmid vectors for defined reprogramming factors is transfected into somatic cells. Plasmid vectors are not integrated into the genome of transfected cells and are gradually lost during reprogramming. This method therefore requires multiple transfection steps. b) Somatic cells can be transfected by episomal vectors expressing defined reprogramming factors. These vectors can replicate themselves autonomously in cells during reprogramming under drug selection and are not integrated into the genome. Upon withdrawal of drug selection, the episomal vectors are lost. c) Adenovirus carrying defined reprogramming factors can be infected into somatic cells to transiently express these factors. This method requires multiple transductions since adenoviral vectors are lost upon cell division. d) The minicircle vector method is based on PhiC31-vector intramolecular recombinant system that allows the bacterial elements of the vector to be degraded in bacteria. Minicircle vector containing only defined reprogramming factors is not degraded and is delivered into somatic cells by nucleofection. This strategy requires multiple transfection steps since minicircle vectors are lost upon cell division. e) Reprogramming using mRNA reprogramming factors have been achieved.

the transduced viral vectors and transgenes are randomly and permanently integrated into the genome of infected somatic cells and remains in the iPSCs. The vector integration into the host genome is a limitation of this technology if it is going to be used in human therapeutic applications due to increased risk of tumor formation (Okita et al., 2007). Approaches to derive transgene-free iPSCs are therefore critical. The first strategy was by using non-integrating (Figure 2) vectors. Efforts have been made to derive iPSCs by repeated plasmid transfections (Gonzalez et al., 2009; Okita et al., 2008) (Figure 2a), adenoviral (Stadtfeld et al., 2008b) (Figure 2b) and episomal vectors (Yu et al., 2009) (Figure 2c). Recently, minicircle vectors (Figure 2d) have been used to generate iPSCs (Jia et al., 2010). Unfortunately, reprogramming with these techniques has extremely low efficiency as compared to integrating viral vectors. Another promising alternative is the use of excisable integrating vectors, allowing for the generation of transgene-free iPSCs. A classical expression-excision system uses vectors with inserts flanked with recognition sites, loxP sites, for Cre-recombinase (Figure 3a). Consequently, DNA is excised upon Cre-recombinase expression in the cells. Cre-loxP-based approaches have been used to reprogram human somatic cells from individuals with Parkinson’s disease by four different vectors (Soldner et al., 2009) or by a single, polycistronic lentiviral vector encoding reprogramming factors (Chang et al., 2009). Though, a potential limitation of Cre-loxP-based approaches is that a long terminal repeat (LTR) will remain after Cre-mediated excision which may interfere with the expression of endogenous genes. An alternative integration-free strategy is based on the piggy-Bac transposon (Figure 3b), a mobile genetic element from insects that integrates into the genome of mammalian cells and, most importantly, can be entirely removed by a transposase. Two research teams generated iPSCs using this system to deliver a single polycistron encoding four reprogramming factors into somatic cells (Woltjen et al., 2009; Yusa et al., 2009). Interestingly, the latest development indicates that gene transfection may not even be needed for the generation of iPSCs and that direct delivery of four recombinant reprogramming proteins that can penetrate the plasma membrane of somatic cells is sufficient (Zhou et al., 2009), or mRNA (Angel & Yanik, 2010; Plews et al., 2010; Warren et al. 2010; Yakoba et al., 2010; Zhou et al., 2009).
Fig. 3. Methods for production of induced pluripotent stem cells (iPSCs) by excisable integrating vectors. Two different methods exist today to generate iPSCs by excisable integrating vectors: by Cre-loxP and Piggy-Bac vectors. a) In the Cre-loxP viral delivery system, defined reprogramming factors are cloned into vectors flanked by recognition sites, loxP sites, for Cre-recombinase. Upon transduction into somatic cells, the loxP site is duplicated and reprogramming factors are stably integrated into the genome flanked by loxP sites. When Cre-recombinase is expressed, the integrated reprogramming factors are excised from the genome but one loxP site is left behind integrated into the genome of iPSCs. b) The Piggy-Bac transposon gene delivery system is based on a mobile genetic element that efficiently integrates into the genome of mammalian cells. When fusion gene encoding defined reprogramming factors in the transposon expression vector as well as transposase expression vector are transfected into somatic cells, the fusion gene is stably integrated into the genome. When transposase is expressed, the integrated genetic material is excised from the genome resulting in transgene- and vector free iPSCs.

The therapeutic application of iPSCs is limited by another concern due to the use of potential oncogenes when iPSCs are produced. C-Myc is an oncogene and as such causes
tumor formation, which has been observed in iPSC-derived chimeric mice (Okita et al., 2007). As a major step towards solving this issue, several studies have demonstrated that mouse and human iPSCs can be derived without C-Myc but the efficiency of reprogramming is reduced (Nakagawa et al., 2008; Wernig et al., 2008b; Yu et al., 2007). Although the oncogenic potential of C-Myc is mostly discussed, Oct4, Sox2 and Klf4 are also associated with multiple types of cancer (Bass et al., 2009; Gidekel et al., 2003; Wei et al., 2006). To circumvent this problem, a recent trend is to avoid the transduction of some of the oncogenes by 1) reprogramming somatic cells which already endogenously express sufficient levels of some of the reprogramming factors (Tsai et al., 2010), 2) replacing one or more reprogramming factors by small molecules like histone deacetylase inhibitor vaporic acid, the DNA methyltransferase inhibitor 5-aza-cytidine, the Wnt signaling component WNT3a, the L-channel calcium channel agonist Bayk8644 (Huangfu et al., 2008a; Huangfu et al., 2008b), or 3) dual inhibition of mitogen activated protein kinase signaling and glycogen synthase kinase-3 (Silva et al., 2008). It has been reported that Sox2 can be replaced by Sox1, Klf4 by Klf2 and c-Myc by N-myc or L-myc indicating that they are not fundamentally required for generation of iPSCs (Yamanaka, 2009). Tet-on™ technology has been used to express exogenously reprogramming factors in presence of Doxycycline. Removal of Doxycycline results in that iPSC colonies that endogenously express pluripotent genes and colonies that are truly reprogrammed remains.

5. Transplanting cells

In order to make cell therapy (route 1 in Figure 1) using iPSCs a reality in medicine many obstacles need to be overcome. Organ transplantation between individuals is complicated due to the limited availability of matched tissues and consequently the requirement for life-long treatment with immunosuppressive drugs that can cause serious side effects. The hope is that iPSCs that are already genetically matched with the patient would circumvent these issues. Another advantage of iPSCs over current transplantation approaches is the opportunity of repairing mutations that cause the disease by homologous recombination, which has not been very successful in adult stem cells due to difficulties in propagating those cells in vitro. In mouse, iPSC technology combined with correction of a known disease-causing mutation has been proven successful. In human autologous cell therapy has been used since the mid 90’s for the treatment of focal cartilage lesions, using the patient’s chondrocytes transplanted into the injured knee (Brittberg et al., 1994), thereby alleviated osteoarthritic symptoms and induction of tissue repair. The cell therapy gives stable long-term results up to 20 years after surgery in some patients but is less successful in others (Lindahl et al., 2003; Peterson et al., 2010). One drawback with this technique is the supply of cells. Large injuries require large amounts of cells, and there is a limit of the size of the biopsies that can be taken out from the patient. Introducing the iPSC technique in such system might improve the process. Since the iPSCs have theoretically an unlimited proliferation capacity, these cells can be used to reach larger quantities of cells. When sufficient numbers have been produced, the iPSCs are differentiated into chondrocytes and transplanted to the lesion. In this case, no biopsy would need to be harvested, since iPSCs can be made from a regular skin fibroblast. Before this somewhat futuristic scenario can come true, rigorous characterization of the iPSC is needed, since these cells, as all stem cells,
can form teratoma in vivo (Fairchild, 2010). The iPSCs have however, been shown to retain their epigenic memory from the tissue from which they originate. It would therefore be easier to differentiate an iPSC to a chondrocyte if the donor cell was a chondrocyte (Kim et al., 2010), and maybe terminally so, thus avoiding risk for teratoma formation. A biopsy would thus be needed, but a relatively small cell harvest could with the iPSC technique result in the treatment of larger injuries. The iPSC procedure could also lead to a therapy-outcome that is more predicted and constant due to that chondrogenic differentiation of iPSC probably result in a more homogeneous cell-population. Since cartilage lacks vascularisation and thus is immunoprivileged the derivation of a universal donor chondrocytes cell line based on the iPSC technology could be an interesting option. If such cells are combined with a suitable matrix scaffold a cartilage regeneration therapy could potentially have a much wider application and be more cost effective than current autologous procedures.

5.1 Directprogramming of somatic cells into another cell type
Switching from one somatic cell type into another cell type, not necessarily via a pluripotent cell state was first demonstrated when fibroblasts formed myofibers after transduction with retroviral vectors expressing the skeletal muscle factor MyoD (Davis et al., 1987). Further, it has been reported that pancreatic acinar cells could be transformed into insulin-producing β cells by overexpression of the pancreatic factors Pdx1, MafA and Ngn3 in vivo (Zhou et al., 2008) as well as that ESCs could be directly differentiated into specific dopamine neurons by overexpression of only one factor, Lmx1 (Friling et al., 2009). These experiments proved that transdifferentiation do not require reprogramming into a pluripotent state, although all such experiments have used some kind of retroviruses and if only virus in itself can contribute to pluripotency as has recently been shown one cannot completely rule out that the switch hasn’t passed via a pluripotent state.

6. Final remarks
To date, clinically valid iPSCs do not yet exist, but are under development worldwide. Some will argue that the complexity of reprogramming is solved by the iPSC technology, however apart from the defined reprogramming factors, retroviruses help in the reprogramming process in an unknown way, and is still inefficient compared to SCNT which argues for that more can be learnt about reprogramming. Also the fact that different combinations of reprogramming factors, or replacement with chemicals, have been used successfully indicates that there exist reprogramming molecules yet to be discovered. Therefore, further investigations are needed to learn more about the molecular mechanisms of iPSCs and how to prevent tumor formation following in vivo transplantation. Awaiting in vivo safety, these techniques offer exciting possibilities for mapping mechanisms of different diseases and screening for patient-specific therapies and drugs. To derive iPSCs from the patient’s own cells following differentiation into the disease-causing cells means recapitulating the disease in a test tube for genomic, proteomic and epigenomic analysis. The iPSC as a human in vitro disease modeling system is a new promising and fast expanding research area.
7. References


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This book documents the increased number of stem cell-related research, clinical applications, and views for the future. The book covers a wide range of issues in cell-based therapy and regenerative medicine, and includes clinical and preclinical chapters from the respected authors involved with stem cell studies and research from around the world. It complements and extends the basics of stem cell physiology, hematopoietic stem cells, issues related to clinical problems, tissue typing, cryopreservation, dendritic cells, mesenchymal cells, neuroscience, endovascular cells and other tissues. In addition, tissue engineering that employs novel methods with stem cells is explored. Clearly, the continued use of biomedical engineering will depend heavily on stem cells, and this book is well positioned to provide comprehensive coverage of these developments.

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