

## Determinants of Pluripotency: From Avian, Rodents, to Primates

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### ABSTRACT

Since mouse embryonic stem (ES) cells was first derived in 1981, the ability of this unprecedented cell type to self-renew and differentiate without limit has revolutionized the discovery tools that are used to study gene functions and development. Furthermore, they have inspired others to hunt for similar cells from other species. The derivation of human ES cells in 1998 has accelerated these discoveries and has also widely provoked public interest, due to both the scientific significance of these cells for human tissue regeneration and the ethical disputes over the use of donated early human embryos. However, this is no longer a barrier, with the recent discovery of methods that can convert differentiated somatic cells into ES-like cells or induced pluripotent stem (iPS) cells, by using defined reprogramming factors. This review attempts to summarize the progresses in the derivation of ES cells (as well as other embryo-derived pluripotent cells) and iPS cells from various species. We will focus on the molecular and biological features of the cells, as well as the different determinants identified thus far to sustain their pluripotency. *J. Cell. Biochem.* 109: 16–25, 2010. © 2009 Wiley-Liss, Inc.

**KEY WORDS:** PLURIPOTENCY; EMBRYONIC STEM CELLS; INDUCED PLURIPOTENT STEM CELLS; SPECIES; GROWTH FACTORS

**S**ir Martin Evans of Cardiff University shared the 2007 Nobel Prize in Physiology or Medicine with the two founders of gene targeting. This marked the highest recognition for mouse embryonic stem (ES) cells [Evans and Kaufman, 1981; Martin, 1981]. Due to their unlimited ability to self-renew and differentiate, this unprecedented cell type has revolutionized the discovery tools used to study gene functions and development. It has also inspired others to hunt for similar cells from many other species. However, it took scientists 17 years to eventually derive human ES cells [Thomson et al., 1998]. The success of Thomson and coworkers was based on their prior successes in the derivation of non-human primate ES cells [Thomson et al., 1995; 1996]. The birth of human ES cells has triggered new waves of discoveries in regenerative biology and in the derivation of ES cells from other species. Meanwhile, it has aroused extensive public interest due to the promise of human ES cells in regenerating human tissues, as well as ethical concerns over the disputable use of donated early human embryos to derive these cells.

To overcome this hurdle, scientists soon started to seek alternative approaches to procuring ES-like cells without using embryos. This was elegantly achieved by Takahashi and Yamanaka in 2006, who discovered that four essential transcription factors that are highly

expressed in ES cells can be used to reprogram somatic cells into an embryonic-like state, which led to the creation of another new pluripotent stem (PS) cell type, known as induced PS (iPS) cells [Takahashi and Yamanaka, 2006]. This new technology has been quickly recapitulated in human [Takahashi et al., 2007; Yu et al., 2007a] and many other species including monkey [Liu et al., 2008], pig [Esteban et al., 2009; Ezashi et al., 2009; Wu et al., 2009], and rat [Li et al., 2009; Liao et al., 2009].

In this review, we will summarize the recent advances in the derivation of ES cell lines and iPS cells lines from various species, as well as other embryo-derived PS cells. We will discuss the key determinants of their pluripotency, focusing mainly on the molecular and biological features of the PS cell lines. The external factors identified thus far to sustain their pluripotency will also be summarized.

### EMBRYONAL CARCINOMA (EC) CELLS

Pluripotency refers to the ability of a cell to not only renew itself but also to differentiate into many other cell types. This ability was first demonstrated in mouse EC cells, which are derived from

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teratocarcinomas. Mouse EC cells can be stably propagated in vitro, while spontaneously differentiating into various cell types that represent all the three embryonic germ (EG) layers [reviewed in Andrews et al., 2005]. Many pluripotency assays established on EC cells, such as pluripotency marker detection and embryoid body (EB) formation in vitro and in vivo (now referred to as teratoma formation), are still widely used today for validation of ES, iPS, and other PS cells [Andrews et al., 2005].

Mouse EC cells can express markers found within the inner cell mass (ICM) of the blastocyst, including stage-specific embryonic antigen-1 (SSEA-1) [Andrews et al., 2005], but not SSEA-3 and SSEA-4. Human EC cells have also been derived from human teratocarcinomas. However, human EC cells express different cell surface markers from mouse EC cells. Human EC cells do not express SSEA-1, but instead express SSEA-3, SSEA-4, and the tumor-related antigens TRA1-60 and TRA1-81 [Andrews et al., 2005]. These markers are not expressed on mouse EC cells.

Although both mouse and human EC cells are karyotypically abnormal due to their tissue source, they continue to accumulate additional karyotypic changes in culture, which most likely contributes to their unlimited proliferation in serum-containing medium independently of growth factors. On the other hand, these abnormalities may also account for their limited developmental potential [reviewed in Andrews et al., 2005]. ES cells can also develop similar karyotypic changes during long-term culture; cells harboring pro-proliferation changes, for example, human ES cells with trisomy chromosome 12 or replication of its short arm, may gain selection advantage over sibling cells [Draper et al., 2004]. Thus, the early studies of EC cells have served as cornerstones for characterizing and understanding the nature of ES cells.

## EMBRYONIC STEM (ES) CELLS

The derivation and characterization of mouse ES cells have set the basic methods and standards for the subsequent derivation of ES cells from other species, including human. Although pluripotency is remarkably consistent for all bona fide ES cells, significant differences have been identified among ES cells derived from various species and some routine tests established on mouse ES cells, such as chimera formation and germline transmission, cannot be applied to human ES cells for ethical reasons. ES cell lines from various species will be discussed in chronological order.

### MOUSE ES CELLS

Mouse ES cells were first derived by culturing the ICM of mouse blastocysts in serum-containing medium on mitotically inactivated mouse embryonic fibroblasts (MEFs) as a feeder layer [Evans and Kaufman, 1981; Martin, 1981]. Morphologically, mouse ES cells are small and round with a large nucleus-to-cytoplasm ratio and prominent nucleoli, highly resembling mouse EC cells. As described above, mouse EC cells have abnormal karyotypes, whereas mouse ES cells have normal karyotype, although it can become unstable in extended culture. Mouse ES cells form small, rounded, or irregular colonies with clear borders. Similar to mouse EC cells, they are

stained positively for alkaline phosphatase and SSEA-1, negatively for SSEA-3, SSEA-4, TRA1-60, and TRA1-81. They also highly express the transcription factors Oct3/4, Nanog, and Sox2 to sustain their pluripotency. Mouse ES cells can be differentiated in vitro into cell types that represent all the three EG layers, through directed differentiation or formation of EBs. They can also form teratomas when injected into immunodeficient mice. Furthermore, they are capable of producing chimeras with germline transmission.

The cytokine leukemia inhibitory factor (LIF) is required to sustain mouse ES cells. LIF binds the gp130 receptor, inducing the dimerization of gp130 and LIF receptors, which activates Janus-associated tyrosine kinases (Jak)/latent signal transducer and activator of transcription factor (Stat3) [Yoshida et al., 1994]. The activation of Stat3 is sufficient to maintain the undifferentiated state of mouse ES cells in the presence of LIF and serum [Matsuda et al., 1999]. The LIF/gp130 receptors can also activate Shp2 tyrosine phosphatase, which then promotes mouse ES cell differentiation by inhibiting Jak/Stat3 signaling and enhancing Ras/Raf/Mek/Erk signaling, thus suppression of the Shp2/Erk pathway promotes ES cell self-renewal [reviewed in Feng, 2007]. Bone morphogenetic proteins (BMPs) were found to be the responsible components in serum to synergize with LIF to support mouse ES cells self-renewal [Ying et al., 2003]. BMP4 acts by inducing *Id1* expression [Ying et al., 2003] and inhibiting the Mapk pathways [Qi et al., 2004].

The efficiency of mouse ES cell derivation appears to be influenced by the genetic background of mouse strains, as ES cells can be reproducibly derived from only a few inbred mouse strains: strain 129 and, less commonly, C57BL/6 [Lederhann and Burki, 1991; Kawase et al., 1994]. Protocol modifications have enabled the derivation of ES cells from non-permissive strains. For example, drug selection to continuously remove differentiated cells has allowed derivation of germline competent ES cell lines from the non-permissive mouse strain CBA [McWhir et al., 1996]. Inhibitors of Erk activity can also help derive germline competent ES cell lines from non-permissive mouse strains [Batlle-Morera et al., 2008]. Supplementing LIF-containing medium with Pluripotin (also called SC-1), a small molecule that inhibits the differentiation-inducing factors RasGAP and Erk1 [Chen et al., 2006], can increase the efficiency of mouse ES cell derivation from refractory strains [Yang et al., 2009].

Recently, a ground state of pluripotency has been proposed based on the fact that mouse ES cells can be derived without need for any growth factors but with only a combination of three inhibitors (3i) SU5402, PD184352, and CHIR99021 that target FGF receptors, Mek, and glycogen synthase kinase 3 (Gsk3), respectively [Ying et al., 2008]. Gsk3 is a key component of the  $\beta$ -catenin degradation complex; inhibition of Gsk3 releases  $\beta$ -catenin, enables it to enter the nucleus, and regulates target genes of the canonical Wnt signaling [Ding et al., 2000]. Nuclear  $\beta$ -catenin forms a complex with T-cell factor/lymphoid enhancer factor (Tcf/Lef) transcription factors, Legless family docking proteins (Bcl9 and Bcl9l), and Pygo family coactivators (Pygo1 and Pygo2) to activate the transcription of target genes such as *c-Myc*, which play important roles in mouse ES cell self-renewal [reviewed in Katoh and Katoh, 2007]. Although the absence of FGF ligands may suffice to replace the inhibitors of FGF receptor and Mek, the presence of a Wnt ligand may be

necessary to substitute for the Gsk3 inhibitor to stimulate Wnt signaling. This argues that the pluripotency of mouse ES cells is not completely in a ground state since it relies on at least some external factor to stimulate Wnt signaling. BMP/LIF and 3i (or 2i when SU5402 is removed) systems can support mouse ES cell pluripotency as long as the differentiation-inducing Erk signaling is repressed either upstream by chemical inhibitors or downstream by LIF and BMP [Ying et al., 2008]. Inhibition of Gsk3 promotes self-renewal by maintaining cell proliferation and preventing neural commitment.

### MONKEY ES CELLS

Monkey ES cells have thus far been derived from rhesus macaques [Thomson et al., 1995], common marmoset [Thomson et al., 1996], and cynomolgus [Suemori et al., 2001]. Monkey ES cells and monkeys offer alternative and sometimes irreplaceable tools to study human development and diseases. Potential therapies to treat human diseases must be pioneered in non-human primates prior to human trials for scientific and ethical reasons. While rodent models are useful for modeling some human diseases, they are not ideal for many others such as neural degenerative diseases. Rodent models often cannot adequately recapitulate the pathology of human neural degenerative diseases.

When cultured on MEF feeders (LIF is not necessary), monkey ES cells can be stably maintained in an undifferentiated state with a normal karyotype. They have the capacity to differentiate into cell types that represent all the three germ layers via *in vitro* differentiation culture conditions, EB, and teratoma formation. Monkey ES cells can produce chimeras [Takada et al., 2002]. However, it remains unknown whether monkey ES cells are capable of germline transmission. Monkey ES cells also express cell surface markers alkaline phosphatase, SSEA-3, SSEA-4, TRA1-60, and TRA1-81, but not SSEA-1, which are identical to the cell surface markers expressed by human ES cells.

### AVIAN ES CELLS

Transgenic chicken can be generated via injection of DNA into oocytes but the technique is tedious and the yield of transgenic animals is low [Love et al., 1994]. By using a combination of growth factors LIF, bFGF, and stem cell factor (SCF), both chicken and quail ES cell lines were successfully derived from stage X blastoderm [Pain et al., 1996]. Avian ES cells have typical ES-like morphological features, with large nucleus-to-cytoplasm ratio with multiple nucleoli, and are alkaline phosphatase positive. Avian ES cells also meet the pluripotency standards set for mouse ES cells, that is, they are SSEA-1 positive, can differentiate to cell lineages representative of the three EG layers *in vitro* via directed differentiation or EB formation, and produce chimeras with germline transmission.

### HUMAN ES CELLS

The advances in culture conditions of human IVF embryos [Gardner et al., 1998] and prior experience with non-human primate ES cells [Thomson et al., 1995, 1996] may have been critical for the success of human ES cell derivation [Thomson et al., 1998]. Human ES cells have characteristic ES cell morphology with large nucleus-to-cytoplasm ratio and multiple prominent nucleoli. They are capable of long-term self-renewal, maintain a normal karyotype (which can

become unstable during extended culture), and can differentiate into cell types that represent all three germ layers via EB or teratoma formation. Human ES cells express the same repertoire of cell surface markers as monkey ES cells. Both monkey and human ES cells grow as flatten, compact colonies, whereas mouse ES cells grow as domed colonies.

Growth factors required for monkey and human ES cells are distinct from those required for mouse ES cells. Monkey and human ES cells do not require LIF, and the Jak/Stat3 pathway does not appear to be responsible for their maintenance [Humphrey et al., 2004; Sumi et al., 2004]. In contrast to the role of BMP4 in supporting mouse ES cell self-renewal [Ying et al., 2003], we have demonstrated that BMPs induce human ES cell differentiation to the trophoblast in serum replacement-containing medium [Xu et al., 2002]. Pera et al. [2004] found that BMP2 induces human ES cell differentiation to the primitive endoderm in serum-containing medium. BMP4 also induces monkey ES cell differentiation to the primitive endoderm [Kobayashi et al., 2008].

Furthermore, although FGF signaling causes mouse ES cell differentiation and TGF $\beta$ /Activin/Nodal signaling is not required for mouse ES cell self-renewal, we [Xu et al., 2005, 2008] and others have shown that these signaling pathways are required to support human ES cell self-renewal [reviewed in Okita and Yamanaka, 2006; Watabe and Miyazono, 2009]. It has been proposed that bFGF can act via a paracrine mechanism by inducing differentiated human ES cells to produce IGF2, which then supports the neighboring undifferentiated ES cells in a pluripotent state [Bendall et al., 2007]. However, we recently found that IGF2 cannot substitute for bFGF to sustain human ES cell culture in the defined medium TeSR1, and bFGF supports human ES cell self-renewal largely by preventing the cells from anoikis, a subtype of apoptosis caused by cell detachment from the matrix [Wang et al., 2009]. Although monkey ES cells share many characteristics with human ES cells, they can be cultured on MEF feeders without bFGF [Yamashita et al., 2006].

### RABBIT ES CELLS

Like other animal models, rabbit ES cells are also an important resource for studying human diseases and for pharmaceutical research. Ji and coworkers first derived rabbit ES cells from the ICM of Japanese white rabbit blastocysts on MEFs and in serum-containing medium [Wang et al., 2007]. Unlike ES cells from any other species, these rabbit ES cells are positive for both mouse and human ES pluripotency markers, including alkaline phosphatase, SSEA-1, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Nanog, Oct4, and Sox2. Rabbit ES cells are karyotypically normal and can be maintained undifferentiated during long-term culture. Furthermore, they can be differentiated into cells that represent all three germ layers, *in vitro* culture conditions, EB or teratoma formation. The same group also demonstrated that FGF, TGF $\beta$ /Activin/Nodal, and Wnt signaling pathways are required to maintain the self-renewal of rabbit ES cells [Wang et al., 2008]. Another group found that LIF signaling is dispensable for the maintenance of self-renewal of rabbit ES cells from this rabbit strain [Honda et al., 2009].

Recently, rabbit ES cells were derived from New Zealand white rabbits [Intawicha et al., 2009]. Contrary to the above reports, these authors suggest that LIF is required for the self-renewal of these ES

cells. However, it should be noted that these ES cells do not share the expression of markers indicative of other LIF-dependent ES cells. They are negative for SSEA-1 and positive for SSEA-4, TRA1-60, and TRA1-81.

The differences between ES cells derived from various strains of rabbits may result from genomic variances between these strains. They may also be caused by differences in the source tissues, derivation, and culture systems, which have been shown to affect the ground state of pluripotency in stem cells derived from mouse and rat embryos [Brons et al., 2007; Tesar et al., 2007; Chou et al., 2008].

### RAT ES CELLS

Rat models are used to study various human diseases, for example, diabetes and hypertension. Thus, derivation of rat ES cells has been expected to provide an important resource for studying these diseases and for pharmaceutical research. However, derivation of bona fide rat ES cells has been difficult, and numerous attempts only produced poorly characterized ES-like cell lines without reconstitution of the germline [reviewed in Vassilieva et al., 2000].

Recently, two groups simultaneously derived germline competent rat ES cells from the ICMs of blastocysts of Dark Agouti [Buehr et al., 2008; Li et al., 2008] and Fischer 344 rats [Buehr et al., 2008]. They used the 3i mouse culture regime described above, that is, CHIR99021 to inhibit Gsk3 activity, PD184352 to inhibit Mek, and SU5402 to inhibit FGF receptors. A 2i regime comprised the Gsk3 inhibitor CHIR99021 and a more potent Mek inhibitor PDO325901 was also used to derive rat ES cells [Buehr et al., 2008; Li et al., 2008]. Rat ES cells derived in either the 3i or the 2i regime were indistinguishable. They display the properties authentic to ES cells, such as large nucleus-to-cytoplasm ratio with prominent nucleoli, long-term self-renewal, pluripotency, EB formation, teratoma formation, and produce chimeras with germline transmission. Rat ES cells express the key pluripotency genes found in mouse ES cells: Nanog, Oct4, Sox2, and Klf4. Furthermore, rat ES cells share a similar expression pattern of cell surface markers as mouse ES cells: SSEA-1 positive, SSEA-4 negative, and GCTM-2 (i.e., TRA1-60) negative [Li et al., 2008].

Like mouse ES cells, LIF/Stat3 signaling plays an important role in rat ES cells to maintain their self-renewal [Buehr et al., 2008; Li et al., 2008]. Moreover, Stat3-overexpressing rat ES cells can be maintained feeder-free in a LIF-containing medium for a short time [Li et al., 2008]. Contrary to mouse ES cells, rat ES cells do not benefit from serum or BMP4 in feeder-free culture [Buehr et al., 2008; Li et al., 2008], suggesting that other factors are required for long-term culture of rat ES cells in feeder-free conditions.

### CANINE ES CELLS

The establishment of ES cells from large animals that model human diseases is of significant importance. Canine ES cell lines have been recently derived from preimplantation-stage embryos [Vaags et al., 2009]. They express Oct3/4, Nanog, Sox2, alkaline phosphatase, SSEA-3, SSEA-4, TRA1-60, and TRA1-81, and a very low level of SSEA-1. Similar to mouse ES cells, canine ES cells require LIF for the maintenance of pluripotency. They can maintain a normal karyotype and morphology typical of other undifferentiated ES cells after multiple passages in vitro. They can also form EBs and

differentiate to multiple cell types. In vivo, canine ES cells gave rise to teratomas comprising cell types that represent all three germ layers. However, the abilities to form chimera and transmit through the germline have yet to be determined. These cells represent the first pluripotent canine ES cell lines and offer the exciting possibility of testing the efficacy and safety of ES cell-based therapies in other large animal models of human disease.

Many attempts have been made to derive ES cells from other mammalian species, including hamster, cow, buffalo, sheep, pig, goat, horse, and cat [reviewed in Tecirlioglu and Trounson, 2007; Vackova et al., 2007; Talbot and Blomberg le, 2008]. However, none of the ES-like cells derived from these species adhere to the criteria set forth by mouse and human ES cell research to be bona fide ES cell lines. These ES-like cell lines cannot be maintained undifferentiated during long-term culture. Appropriate culture conditions need to be elucidated. Furthermore, thorough characterization of their pluripotency markers and developmental potential is necessary. Undoubtedly, derivation of bona fide ES cell lines from these species will be remarkable to understanding the early development of these species as well as their gene functions during development and tissue regeneration. These ES cells can also serve as additional tools for developing and testing potential therapies for the treatment of human diseases.

## OTHER EMBRYO-DERIVED PS CELLS

### EMBRYONIC GERM (EG) CELLS

EG cells are PS cells derived from primordial germ (PG) cells. PG cells are progenitors of adult gametes, which diverge from the somatic lineage between late embryonic to early fetal development. EG cells were first derived from mouse PG cells, using MEFs, SCF, LIF, and bFGF [Matsui et al., 1992; Resnick et al., 1992]. EG cells have also been derived from human [Shablott et al., 1998], chicken [Park and Han, 2000], and pig [Shim et al., 1997]. As ES cells, EG cells demonstrate long-term self-renewal ability and differentiate in vitro to form EBs, containing cells that represent all three germ layers and intermediate progenitor cells. They can also form teratomas in immunodeficient mice. Furthermore, mouse, pig, and chicken EG cells have also been shown to contribute to formation of chimeras with germline transmission. Importantly, EG cells demonstrate normal and stable karyotypes as well as normal patterns of genomic imprinting, including X-inactivation within certain passages in vitro [Kerr et al., 2006].

### EPIBLAST STEM CELLS (EpiSCs)

The dramatic differences between the signaling pathways required for maintenance of pluripotency of mouse and human ES cells have prompted scientists to ask whether mouse ES cells could be derived under the same conditions used for human ES cells. However, the ICMs from mouse and rat blastocysts grown in chemically defined medium, containing activin A and bFGF, undergo rapid differentiation and never give rise to pluripotent ES cell lines [Brons et al., 2007]. This medium is sufficient for the long-term maintenance of human ES cells [Vallier et al., 2005]. Interestingly, if late epiblast layer of postimplantation mouse or rat embryos is cultured in the

same medium, PS cell can be derived [Brons et al., 2007; Tesar et al., 2007]. These PS cells were designated EpiSCs, due to their origin. EpiSCs express typical stem cell markers such as Oct3/4, Nanog, and SSEA-1, but no Rex1 and little Gbx2. They are capable of differentiating into tissues that represent all three germ layers, as determined by EB and teratoma formation, and in vitro differentiation culturing conditions. However, EpiSCs fail to contribute to chimera formation.

Mouse and rat EpiSCs differ significantly from mouse and rat ES cells but share key features with human ES cells [Brons et al., 2007; Tesar et al., 2007]. They form flat and compact colonies that differ from the domed or rounded colonies from mouse or rat ES cells. Furthermore, like human ES cells [Xu et al., 2002; Pera et al., 2004], BMPs induce EpiSCs to differentiate into primitive endoderm and trophectoderm.

### BFGF, ACTIVIN, AND BIO-DERIVED STEM CELLS (FAB-SCs)

PS cells have also been derived from mouse blastocysts cultured on MEFs in the presence of bFGF, ActivinA, 6-bromoindirubin-3'-oxime (BIO), and LIF-blocking antibody [Chou et al., 2008]. BIO is an inhibitor of Gsk3 [Sato et al., 2004]. These stem cells are designated as FAB-SCs, based on the chemicals used to derive them. Distinct from both mouse EpiSCs and ES cells, FAB-SCs can be maintained in the absence of LIF and BMP4. They express several markers of pluripotency, including Oct3/4, Nanog, Sox2, and SSEA-1. Unexpectedly, these cells cannot differentiate into tissues representing the three germ layers. EBs formed by FAB-SCs are small and cannot expand. Furthermore, FAB-SCs do not form teratomas after injection into SCID mice. However, transient stimulation with LIF and BMP4 changes the developmental potential of FAB-SCs. The stimulated FAB-SCs can generate teratomas when injected into SCID mice and contribute to the germline in chimeric mice. These attributes are lost if LIF and BMP4 are withdrawn.

Comparison of the gene expression profiles of mouse ES cells, EpiSCs, and FAB-SCs suggests that each of these PS cell lines are distinct and may represent different metastable epigenetic states that are determined by the tissue of origin and the growth factor milieu [Chou et al., 2008]. FAB-SCs may represent ES-like cells that display partial pluripotency, whereas full pluripotency can be attained upon alteration of their growth factor regime. Thus, FAB-SCs display a high degree of plasticity, suggesting that growth factor signaling may play an important role in reprogramming, which is worth further investigation.

## INDUCED PLURIPOTENT STEM (iPS) CELLS

iPS cells are generated by reprogramming somatic cells back to pluripotent state with defined reprogramming factors. Since the first derivation of mouse iPS cells by the Yamanaka group [Takahashi and Yamanaka, 2006], iPS cells have been derived from various species and tissues with a variety of methods, which have been summarized in many reviews. We will mainly introduce and compare the features of iPS cells derived from several species.

### MOUSE IPS CELLS

MEFs and mouse adult tail fibroblasts were the first source of somatic cells used for iPS cell derivation via retroviral transduction to express the reprogramming factors Oct4, Sox2, Klf4, and c-Myc, and MEFs were also used as feeders in the derivation and maintenance of mouse iPS cells [Takahashi and Yamanaka, 2006]. The iPS cells exhibit the same features as mouse ES cells, including staining positive for alkaline phosphatase and SSEA-1. They can differentiate in vitro into all three germ layers and can form teratomas when injected into SCID mice. Furthermore, they can contribute to the germline in chimeric mice [Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007].

### HUMAN IPS CELLS

Two groups independently derived the first human iPS cell lines by using two partially different combinations of reprogramming factors. Thomson group used OCT4, SOX2, NANOG, and LIN28 via lentiviral transduction [Yu et al., 2007b], whereas Yamanaka group used the reprogramming factors and retroviral transduction [Takahashi et al., 2007] as used in the mouse iPS cells [Takahashi and Yamanaka, 2006]. Human iPS cells are derived on MEF feeders. They are cultured under the same conditions and express the same cell surface markers as human ES cells. Human iPS cells can also be differentiated in vitro into tissues represent all three germ layers and form teratomas when injected into immunodeficient mice.

### METHODOLOGICAL IMPROVEMENT

Since these groundbreaking reports, iPS cells have been generated from various tissues such as keratinocytes, B lymphocytes, bone marrow cells, liver cells, neural stem cells, and meningiocytes [reviewed in Feng et al., 2009]. Pharmaceutical inhibitors that specifically target epigenetic modifiers or pluripotency regulators can be used to replace one or more viral factors, or enhance reprogramming efficiency [reviewed in Feng et al., 2009]. Other recent studies have focused on generating iPS cells using virus-free methods. To prevent transgene integration in the genome, adenoviral transduction [Stadtfeld et al., 2008], transient transfection [Okita et al., 2008], nonintegrating oriP/EBNA1 episomal vectors [Yu et al., 2009], and the *piggyBac* (PB) transposon gene-delivery systems [Kaji et al., 2009; Woltjen et al., 2009; Yusa et al., 2009] have been used for reprogramming mouse cells. Moreover, iPS cells can be generated from mouse and human differentiated cells through transduction of recombinant OCT4, SOX2, KLF4, and c-MYC proteins [Bru et al., 2008; Zhou et al., 2009]. More recently, it has been shown that iPS cells can also be derived and maintained in defined mTeSR1 medium [Ludwig et al., 2006] (Stem Cell Technologies, Vancouver, Canada) from human adipose stem cells with the Yamanaka factors via lentiviral transduction [Sun et al., 2009].

### RAT IPS CELLS

Rat iPS cells were generated from rat primary skin fibroblasts and bone marrow cells, in human ES cell culture conditions [Liao et al., 2009]. Although these cells were capable of differentiation in vitro into all three germ layers and could form teratomas, they could not form chimeras, suggesting that these rat iPS cells may be more

closely related to rat EpiSCs than rat ES cells. However, rat iPS cells generated from rat liver cells by using the 2i regime supplemented with LIF can differentiate in vitro into all three germ layers, form teratomas, and chimeras [Li et al., 2009].

### MONKEY IPS CELLS

Monkey iPS cells have been generated from adult rhesus monkey fibroblasts [Liu et al., 2008]. These iPS cells shared the characteristics of monkey ES cells, including positive staining for alkaline phosphatase, SSEA-4, TRA1-60, and TRA1-81. Monkey iPS cells can also be differentiated into all three germ layers, via directed in vitro differentiation, EB formation, and teratoma formation. It remains to be determined, though, if monkey iPS cells can form chimeras and contribute to the germline.

### PORCINE IPS CELLS

Even though derivation of porcine ES cells has not yet been reported, iPS cells have been derived from a variety of tissues in pigs, and they behave differently depending on the culture conditions. Porcine iPS cells derived from embryonic fibroblasts in serum-containing medium morphologically resemble human ES cells with flatten, compact colonies that stain positively for alkaline phosphatase, SSEA-4, Nanog, and Rex1 [Esteban et al., 2009]. Porcine iPS cells derived from primary ear fibroblasts or bone marrow cells and in human ES cell medium without bFGF also express human ES cell surface and nuclear markers [Wu et al., 2009]. However, iPS cells generated from porcine fetal fibroblasts in human ES cell medium supplemented with BFGF stain positive for alkaline phosphatase and SSEA-1, and negative for SSEA-3, SSEA-4, TRA1-60, and TRA1-81 [Ezashi et al., 2009], which is very unusual compared to other PS cells cultured in human ES cell medium. Nevertheless, all these iPS cells can differentiate into cells representing the three germ layers via EB and teratoma formation.

TABLE I. Cell Surface Markers Expressed on Pluripotent Stem Cell Lines

Stem cell line	AP	SSEA-1	SSEA-3	SSEA-4	TRA1-60	TRA1-81	References
Mouse EC cells	+	+	-	-	-	-	Andrews et al. [2005]
Human EC cells	+	-	+	+	+	+	Andrews et al. [2005]
Mouse ES cells	+	+	-	-	-	-	Evans and Kaufman [1981] and Martin [1981]
Rat ES cells	+	+	-	-	-	-	Buehr et al. [2008] and Li et al. [2008]
Avian ES cells	+	+					Pain et al. [1996]
Canine ES cells	+	Low	+	+	+	+	Vaags et al. [2009]
Monkey ES cells	+	-	+	+	+	+	Thomson et al. [1995, 1996]
Human ES cells	+	-	+	+	+	+	Thomson et al. [1998]
Japanese white rabbit ES cells	+	+	+	+	+	+	Honda et al. [2009] and Wang et al. [2007]
New Zealand white rabbit ES cells	+	-	+	+	+	+	Honda et al. [2009] and Intawicha et al. [2009]
Mouse EG cells	+	+					Matsui et al. [1992] and Resnick et al. [1992]
Human EG cells	+	-	+	+	+	+	Shablott et al. [1998]
Porcine EG cells	+						Shim et al. [1997]
Chicken EG cells	+	+					Park and Han [2000]
Mouse EpiSCs	+	+					Brons et al. [2007] and Tesar et al. [2007]
Rat EpiSCs	+	+					Brons et al. [2007]
Mouse FAB-SCs	+	+					Chou et al. [2008]
Mouse iPS cells	+	+	-	-	-	-	Takahashi and Yamanaka [2006]
Human iPS cells	+	-	+	+	+	+	Takahashi et al. [2007] and Yu et al. [2007b]
Rat iPS cells (in 2i regime, LIF)	+	+	-	-	-	-	Li et al. [2009]
Rat iPS cells (in human ES cell medium)	+	-	+	+	+	+	Liao et al. [2009]
Monkey iPS cells	+	-	+	+	+	+	Liu et al. [2008]
Porcine iPS cells (in serum)	+		+	+	+	+	Esteban et al. [2009]
Porcine iPS cells (in human ES cell medium)	+		+	+	+	+	Ezashi et al. [2009] and Wu et al. [2009]

AP, alkaline phosphatase; Blank, not tested.

## SUMMARY

### CELL SURFACE MARKERS

As summarized in Table I, all the PS cells from the listed species are positive for alkaline phosphatase. However, there is a clear difference in expression of the other cell surface markers between PS cells from rodents and primates. Mouse EC, ES, EG, and iPS cells, and rat ES and iPS (when cultured in mouse ES cell medium) cells are all positive for SSEA-1, and negative for SSEA-3, SSEA-4, TRA1-60, and TRA1-81. In contrast, monkey ES and iPS cells, and human EC, ES, EG, and iPS cells are all positive for SSEA-3 (not tested for monkey iPS cells), SSEA-4, TRA1-60, and TRA1-81, but negative for SSEA-1.

Porcine iPS cells can express primate ES cell surface markers (the rodent marker SSEA-1 was not tested) [Ezashi et al., 2009; Wu et al., 2009]. But surprisingly, they can also express rodent ES cell surface markers when derived in human ES cell medium with bFGF [Ezashi et al., 2009]. Interestingly, Japanese white rabbit ES cells express both the rodent and primate markers [Wang et al., 2007; Honda et al., 2009], whereas New Zealand white rabbit ES cells only express the primate markers (SSEA-3 was not tested) [Honda et al., 2009; Intawicha et al., 2009]. In addition, the expression pattern of rat iPS cells can change depending on the culture conditions. They express the rodent marker SSEA-1 when cultured in mouse ES cell medium [Li et al., 2009], and the primate markers in human ES cell medium [Liao et al., 2009]. Avian ES cells, the only reported bona fide PS cells derived from non-mammals, express the rodent marker SSEA-1 (the primate markers were not tested) [Pain et al., 1996].

### DIFFERENTIATION ABILITIES

We also compared the differentiation ability of the PS cells from various species (Table II). All PS cells, no matter which species they are derived from, can form EB and teratomas, except mouse FAB-SCs which only gain such ability after stimulation with the mouse ES

TABLE II. Differentiation Ability of Pluripotent Stem Cell Lines

Stem cell line	EB	Teratoma	Chimera	Germline transmission	References
Mouse EC cells	+	+			Andrews et al. [2005]
Human EC cells	+	+			Andrews et al. [2005]
Mouse ES cells	+	+	+	+	Evans and Kaufman [1981] and Martin [1981]
Rat ES cells	+	+	+	+	Buehr et al. [2008] and Li et al. [2008]
Avian ES cells	+	+	+	+	Pain et al. [1996]
Canine ES cells	+	+			Vaags et al. [2009]
Monkey ES cells	+	+	+		Cibelli et al. [2002], Suemori et al. [2001], Thomson et al. [1995, 1996], Takada et al. [2002]
Human ES cells	+	+			Thomson et al. [1998]
Japanese white rabbit ES cells	+	+			Honda et al. [2008] and Wang et al. [2007]
New Zealand white rabbit ES cells	+	+			Intawicha et al. [2009]
Mouse EG cells	+	+	+	+	Matsui et al. [1992] and Resnick et al. [1992]
Human EG cells	+	+			Shamblott et al. [1998]
Porcine EG cells	+	+	+	+	Shim et al. [1997]
Chicken EG cells	+	+	+	+	Park and Han [2000]
Mouse EpiSCs	+	+	-	-	Brons et al. [2007] and Tesar et al. [2007]
Rat EpiSCs	+	+	-	-	Brons et al. [2007]
Mouse FAB-SCs	-	-	-	-	Chou et al. [2008]
Mouse FAB-SCs stimulated with LIF, BMP4	+	+	+	+	Chou et al. [2008]
Mouse iPS cells	+	+	+	+	Maherali et al. [2007], Okita et al. [2007], Takahashi and Yamanaka [2006], and Wernig et al. [2007]
Human iPS cells	+	+			Takahashi et al. [2007] and Yu et al. [2007b]
Rat iPS cells (in 2i regime, LIF)	+	+	+	+	Li et al. [2009]
Rat iPS cells (in human ES cell medium)	+	+	-	-	Liao et al. [2009]
Monkey iPS cells	+	+			Liu et al. [2008]
Porcine iPS cells (in serum)	+	+			Esteban et al. [2009]
Porcine iPS cells (in human ES cell medium)	+	+			Ezashi et al. [2009] and Wu et al. [2009]

cell self-renewal factors LIF and BMP4 [Chou et al., 2008]. For ethical reasons, chimera formation and germline transmission abilities cannot be tested for human PS cells. Mouse EC, ES, iPS, FAB-SCs (after stimulation with LIF and BMP4), rat ES and iPS (when cultured in mouse ES cell medium), and avian ES cells can all form chimeras and transmit through germline. However, it remains to be tested whether rabbit ES cells and porcine iPS cells have such abilities.

**GROWTH FACTOR REQUIREMENTS**

At least five signaling pathways initiated by LIF, BMPs, bFGF, TGFβ/Activin/Nodal, and Wnts, respectively, have been widely recognized to regulate pluripotency of PS cells (Table III). LIF and BMP4 can sustain mouse ES and iPS cell self-renewal, whereas only LIF, but not BMPs, can support self-renewal of rat ES and iPS cells. LIF may also sustain the self-renewal of New Zealand white rabbit and avian ES cells. LIF has been used as a self-renewal factor for ES cell

TABLE III. Growth Factors Required for the Maintenance of Pluripotency

Stem cell line	LIF	BMP4	bFGF	TGFβ/Activin/Nodal	Wnt	Others	References
Mouse EC cells							Andrews et al. [2005]
Human EC cells							Andrews et al. [2005]
Mouse ES cells	+	+	-	-	+		Evans and Kaufman [1981] and Martin [1981]
Rat ES cells	+	-	-	-	+		Buehr et al. [2008] and Li et al. [2008]
Avian ES cells	+		+			SCF	Pain et al. [1996]
Canine ES cells							Vaags et al. [2009]
Monkey ES cells	-	-	+	+			Cibelli et al. [2002], Suemori et al. [2001], Thomson et al. [1995, 1996]
Human ES cells	-	-	+	+	+	IGF2	Sato et al. [2004] and Vallier et al. [2005]
Japanese white rabbit ES cells	-		+	+	+		Honda et al. [2008] and Wang et al. [2008]
New Zealand white rabbit ES cells	+						Intawicha et al. [2009]
Mouse EG cells	+		+			SCF	Matsui et al. [1992] and Resnick et al. [1992]
Human EG cells	+		+			SCF, Forskolin	Shamblott et al. [1998]
Porcine EG cells	+		+			SCF	Shim et al. [1997]
Chicken EG cells	+		+			SC, IL-11, IGF-1	Park and Han [2000]
Mouse EpiSCs	-	-	+	+			Brons et al. [2007] and Tesar et al. [2007]
Rat EpiSCs	-	-	+	+			Brons et al. [2007]
Mouse FAB-SCs	-	-	+	+	+		Chou et al. [2008]
Mouse iPS cells	+	+	-	-			Takahashi and Yamanaka [2006]
Human iPS cells	-	-	+	+			Takahashi et al. [2007] and Yu et al. [2007b]
Rat iPS cells (in 2i regime and LIF)	+	-	-	-	+		Li et al. [2009]
Rat iPS cells (in human ES cell medium)	-	-	+	+			Liao et al. [2009]
Monkey iPS cells	-	-	+	+			Liu et al. [2008]
Porcine iPS cells (in serum)							Esteban et al. [2009]
Porcine iPS cells (in human ES cell medium)			+				Ezashi et al. [2009]

derivation from many species, often empirically based on its ability to support mouse ES cell culture. However, it is not required for human ES cells after careful dissection of the growth factor requirements [Daheron et al., 2004], nor for Japanese white rabbit ES cells [Honda et al., 2009].

bFGF and TGF $\beta$ /Activin/Nodal can support self-renewal of human and monkey ES and iPS cells, Japanese white rabbit ES cells, mouse and rat EpiSCs, mouse FAB-SCs, and rat iPS cells (when cultured in human ES cell medium). It has been obvious now that bFGF and TGF $\beta$ /Activin/Nodal can modify the pluripotency ground state of mouse and rat PS cells. Whether alternative use of the LIF/BMP and bFGF/TGF $\beta$  systems can modify the ground state of other PS cells is intriguing and needs to be further explored. Interestingly, Wnt signaling appears to be beneficial to all the tested PS cells by promoting their proliferation among other activities.

## CONCLUDING REMARKS

The rapidly increasing number of PS cells has caused an explosion of literature. Although many articles have reviewed the progresses in these exciting fields, specific summary of PS cells derived from various species has been rare to date. This review has attempted to tackle this task by introducing a variety of PS cells including EC, ES, EG cells, Epi-SCs, FAB-SCs, and iPS cells derived from various species. We have thoroughly compared their cell surface markers, differentiation abilities, and growth factor requirements. However, we did not include technological advancements in culture and lineage-specific differentiation of PS cells, neither progress in translational research of the cells, as these contents have been covered in many other existing reviews, by us [Lin and Xu, In press] and others. We hope that this specific review will help update our knowledge and promote our understanding of PS cells and realization of their promise in biological research and regenerative therapies.

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## REFERENCES

Andrews PW, Matin MM, Bahrami AR, Damjanov I, Gokhale P, Draper JS. 2005. Embryonic stem (ES) cells and embryonal carcinoma (EC) cells: Opposite sides of the same coin. *Biochem Soc Trans* 33:1526–1530.

Battle-Morera L, Smith A, Nichols J. 2008. Parameters influencing derivation of embryonic stem cells from murine embryos. *Genesis* 46:758–767.

Bendall SC, Stewart MH, Menendez P, George D, Vijayaragavan K, Werbowetski-Ogilvie T, Ramos-Mejia V, Rouleau A, Yang J, Bosse M, Lajoie G, Bhatia M. 2007. IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. *Nature* 448:1015–1021.

Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM, Howlett SK, Clarkson A, Ahrlund-Richter L, Pedersen RA, Vallier

L. 2007. Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* 448:191–195 [Epub June 27, 2007].

Bru T, Clarke C, McGrew MJ, Sang HM, Wilmut I, Blow JJ. 2008. Rapid induction of pluripotency genes after exposure of human somatic cells to mouse ES cell extracts. *Exp Cell Res* 314:2634–2642 [Epub May 29, 2008].

Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. *Cell* 135:1287–1298.

Chen S, Do JT, Zhang Q, Yao S, Yan F, Peters EC, Scholer HR, Schultz PG, Ding S. (2006) Self-renewal of embryonic stem cells by a small molecule. *Proc Natl Acad Sci USA* 103:17266–17271 [Epub November 6, 2006].

Chou YF, Chen HH, Eijpe M, Yabuuchi A, Chenoweth JG, Tesar P, Lu J, McKay RD, Geijsen N. 2008. The growth factor environment defines distinct pluripotent ground states in novel blastocyst-derived stem cells. *Cell* 135:449–461.

Cibelli JB, Grant KA, Chapman KB, Cunniff K, Worst T, Green HL, Walker SJ, Gutin PH, Vilner L, Tabar V, Dominko T, Kane J, Wettstein PJ, Lanza RP, Studer L, Vrana KE, West MD. 2002. Parthenogenetic stem cells in nonhuman primates. *Science* 295:819.

Daheron L, Opitz SL, Zaehres H, Lensch MW, Andrews PW, Itskovitz-Eldor J, Daley GQ. 2004. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. *Stem Cells* 22:770–778.

Ding VW, Chen RH, McCormick F. 2000. Differential regulation of glycogen synthase kinase 3 $\beta$  by insulin and Wnt signaling. *J Biol Chem* 275:32475–32481.

Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, Meisner L, Zwaka TP, Thomson JA, Andrews PW. 2004. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol* 22:53–54.

Esteban MA, Xu J, Yang J, Peng M, Qin D, Li W, Jiang Z, Chen J, Deng K, Zhong M, Cai J, Lai L, Pei D. 2009. Generation of induced pluripotent stem cell lines from Tibetan miniature pig. *J Biol Chem* 284:17634–17640 [Epub April 17, 2009].

Evans MJ, Kaufman MH. 1981. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292:154–156.

Ezashi T, Telugu BP, Alexenko AP, Sachdev S, Sinha S, Roberts RM. 2009. Derivation of induced pluripotent stem cells from pig somatic cells. *Proc Natl Acad Sci USA* 106:10993–10998 [Epub June 18, 2009].

Feng GS. 2007. Shp2-mediated molecular signaling in control of embryonic stem cell self-renewal and differentiation. *Cell Res* 17:37–41.

Feng B, Ng JH, Heng JC, Ng HH. 2009. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. *Cell Stem Cell* 4:301–312.

Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. 1998. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil Steril* 69:84–88.

Honda A, Hirose M, Inoue K, Ogonuki N, Miki H, Shimozawa N, Hatori M, Shimizu N, Murata T, Hirose M, Katayama K, Wakisaka N, Miyoshi H, Yokoyama KK, Sankai T, Ogura A. 2008. Stable embryonic stem cell lines in rabbits: Potential small animal models for human research. *Reprod Biomed Online* 17:706–715.

Honda A, Hirose M, Ogura A. 2009. Basic FGF and Activin/Nodal but not LIF signaling sustain undifferentiated status of rabbit embryonic stem cells. *Exp Cell Res* 315:2033–2042 [Epub February 6, 2009].

Humphrey RK, Beattie GM, Lopez AD, Bucay N, King CC, Firpo MT, Rose-John S, Hayek A. 2004. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. *Stem Cells* 22:522–530.

Intawicha P, Ou YW, Lo NW, Zhang SC, Chen YZ, Lin TA, Su HL, Guu HF, Chen MJ, Lee KH, Chiu YT, Ju JC. 2009. Characterization of embryonic stem cell lines derived from New Zealand white rabbit embryos. *Cloning Stem Cells* 11:27–38.

- Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. 2009. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* 458:771–775 [Epub March 1, 2009].
- Katoh M, Katoh M. 2007. WNT signaling pathway and stem cell signaling network. *Clin Cancer Res* 13:4042–4045.
- Kawase E, Suemori H, Takahashi N, Okazaki K, Hashimoto K, Nakatsuji N. 1994. Strain difference in establishment of mouse embryonic stem (ES) cell lines. *Int J Dev Biol* 38:385–390.
- Kerr CL, Gearhart JD, Elliott AM, Donovan PJ. 2006. Embryonic germ cells: When germ cells become stem cells. *Semin Reprod Med* 24:304–313.
- Kobayashi M, Takada T, Takahashi K, Noda Y, Torii R. 2008. BMP4 induces primitive endoderm but not trophoblast in monkey embryonic stem cells. *Cloning Stem Cells* 10:495–502.
- Ledermann B, Burki K. 1991. Establishment of a germ-line competent C57BL/6 embryonic stem cell line. *Exp Cell Res* 197:254–258.
- Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL. 2008. Germline competent embryonic stem cells derived from rat blastocysts. *Cell* 135:1299–1310.
- Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T, Hao E, Hayek A, Deng H, Ding S. 2009. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. *Cell Stem Cell* 4:16–19 [Epub December 18, 2008].
- Liao J, Cui C, Chen S, Ren J, Chen J, Gao Y, Li H, Jia N, Cheng L, Xiao H, Xiao L. 2009. Generation of induced pluripotent stem cell lines from adult rat cells. *Cell Stem Cell* 4:11–15 [Epub December 18, 2008].
- Lin G, Xu RH. In press. Progresses and challenges in optimization of human pluripotent stem cell culture. *Curr Stem Cell Res Ther*.
- Liu H, Zhu F, Yong J, Zhang P, Hou P, Li H, Jiang W, Cai J, Liu M, Cui K, Qu X, Xiang T, Lu D, Chi X, Gao G, Ji W, Ding M, Deng H. 2008. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell* 3:587–590.
- Love J, Gribbin C, Mather C, Sang H. 1994. Transgenic birds by DNA microinjection. *Biotechnology (N Y)*. 12:60–63.
- Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conard KR, Piekarczyk MS, Llanas RA, Thomson JA. 2006. Derivation of human embryonic stem cells in defined conditions. *Nat Biotechnol*. 24:185–187.
- Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. 2007. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 1:55–70.
- Martin GR. 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* 78:7634–7638.
- Matsuda T, Nakamura T, Nakao K, Arai T, Katsuki M, Heike T, Yokota T. 1999. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. *EMBO J* 18:4261–4269.
- Matsui Y, Zsebo K, Hogan BL. 1992. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* 70:841–847.
- McWhir J, Schnieke AE, Ansell R, Wallace H, Colman A, Scott AR, Kind AJ. 1996. Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background. *Nat Genet* 14:223–226.
- Okita K, Yamanaka S. 2006. Intracellular signaling pathways regulating pluripotency of embryonic stem cells. *Curr Stem Cell Res Ther* 1:103–111.
- Okita K, Ichisaka T, Yamanaka S. 2007. Generation of germline-competent induced pluripotent stem cells. *Nature* 448:313–317 [Epub June 6, 2007].
- Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. 2008. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 322:949–953 [Epub October 9, 2008].
- Pain B, Clark ME, Shen M, Nakazawa H, Sakurai M, Samarut J, Etches RJ. 1996. Long-term in vitro culture and characterisation of avian embryonic stem cells with multiple morphogenetic potentialities. *Development* 122:2339–2348.
- Park TS, Han JY. 2000. Derivation and characterization of pluripotent embryonic germ cells in chicken. *Mol Reprod Dev* 56:475–482.
- Pera MF, Andrade J, Houssami S, Reubinoff B, Trounson A, Stanley EG, Ward-van Oostwaard D, Mummery C. 2004. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. *J Cell Sci* 117:1269–1280.
- Qi X, Li TG, Hao J, Hu J, Wang J, Simmons H, Miura S, Mishina Y, Zhao GQ. 2004. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. *Proc Natl Acad Sci USA* 101:6027–6032 [Epub April 9, 2004].
- Resnick JL, Bixler LS, Cheng L, Donovan PJ. 1992. Long-term proliferation of mouse primordial germ cells in culture. *Nature* 359:550–551.
- Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. 2004. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med* 10:55–63 [Epub December 21, 2003].
- Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ, Blumenthal PD, Huggins GR, Gearhart JD. 1998. Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA* 95:13726–13731.
- Shim H, Gutierrez-Adan A, Chen LR, BonDurant RH, Behboodi E, Anderson GB. 1997. Isolation of pluripotent stem cells from cultured porcine primordial germ cells. *Biol Reprod* 57:1089–1095.
- Solter D, Knowles BB. 1978. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc Natl Acad Sci USA* 75:5565–5569.
- Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. 2008. Induced pluripotent stem cells generated without viral integration. *Science* 322:945–949 [Epub September 25, 2008].
- Suemori H, Tada T, Torii R, Hosoi Y, Kobayashi K, Imahie H, Kondo Y, Iritani A, Nakatsuji N. 2001. Establishment of embryonic stem cell lines from cynomolgus monkey blastocysts produced by IVF or ICSI. *Dev Dyn* 222:273–279.
- Sumi T, Fujimoto Y, Nakatsuji N, Suemori H. 2004. STAT3 is dispensable for maintenance of self-renewal in nonhuman primate embryonic stem cells. *Stem Cells* 22:861–872.
- Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry AM, Robbins RC, Longaker MT, Wu JC. 2009. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A*. 106:15720–15725.
- Takada T, Suzuki Y, Kondo Y, Kadota N, Kobayashi K, Nito S, Kimura H, Torii R. 2002. Monkey embryonic stem cell lines expressing green fluorescent protein. *Cell Transplant* 11:631–635.
- Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676 [Epub August 10, 2006].
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861–872.
- Talbot NC, Blomberg le A. 2008. The pursuit of ES cell lines of domesticated ungulates. *Stem Cell Rev* 4:235–254 [Epub July 9, 2008].
- Tecirlioglu RT, Trounson AO. 2007. Embryonic stem cells in companion animals (horses, dogs and cats): Present status and future prospects. *Reprod Fertil Dev* 19:740–747.
- Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, Gardner RL, McKay RD. 2007. New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* 448:196–199 [Epub June 27, 2007].

- Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP. 1995. Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci USA* 92:7844–7848.
- Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP. 1996. Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. *Biol Reprod* 55:254–259.
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. 1998. Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145–1147.
- Vaags AK, Rosic-Kablur S, Gartley CJ, Zheng YZ, Chesney A, Villagomez DA, Kruth SA, Hough MR. 2009. Derivation and characterization of canine embryonic stem cell lines with in vitro and in vivo differentiation potential. *Stem Cells* 27:329–340.
- Vackova I, Ungrova A, Lopes F. 2007. Putative embryonic stem cell lines from pig embryos. *J Reprod Dev* 53:1137–1149.
- Vallier L, Alexander M, Pedersen RA. 2005. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. *J Cell Sci* 118:4495–4509.
- Vassilieva S, Guan K, Pich U, Wobus AM. 2000. Establishment of SSEA-1- and Oct-4-expressing rat embryonic stem-like cell lines and effects of cytokines of the IL-6 family on clonal growth. *Exp Cell Res* 258:361–373.
- Wang S, Tang X, Niu Y, Chen H, Li B, Li T, Zhang X, Hu Z, Zhou Q, Ji W. 2007. Generation and characterization of rabbit embryonic stem cells. *Stem Cells* 25:481–489 [Epub October 12, 2006].
- Wang S, Shen Y, Yuan X, Chen K, Guo X, Chen Y, Niu Y, Li J, Xu RH, Yan X, Zhou Q, Ji W. 2008. Dissecting signaling pathways that govern self-renewal of rabbit embryonic stem cells. *J Biol Chem* 283:35929–35940.
- Wang X, Lin G, Martins-Taylor K, Zeng H, Xu RH. 2009. Inhibition of caspase-mediated anoikis is critical for bFGF-sustained culture of human pluripotent stem cells. *J Biol Chem* 16:16.
- Watabe T, Miyazono K. 2009. Roles of TGF-beta family signaling in stem cell renewal and differentiation. *Cell Res* 19:103–115.
- Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. 2007. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448:318–324 [Epub June 6, 2007].
- Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hamalainen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A. 2009. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* 458:766–770 [Epub March 1, 2009].
- Wu Z, Chen J, Ren J, Bao L, Liao J, Cui C, Rao L, Li H, Gu Y, Dai H, Zhu H, Teng X, Cheng L, Xiao L. 2009. Generation of pig-induced pluripotent stem cells with a drug-inducible system. *J Mol Cell Biol* 3:3.
- Xu RH, Chen X, Li DS, Li R, Addicks GC, Glennon C, Zwaka TP, Thomson JA. 2002. BMP4 initiates human embryonic stem cell differentiation totipoblast. *Nat Biotechnol* 20:1261–1264 [Epub November 11, 2002].
- Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. 2005. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat Methods* 2:185–190.
- Xu RH, Sampsel-Barron TL, Gu F, Root S, Peck RM, Pan G, Yu J, Antosiewicz-Bourget J, Tian S, Stewart R, Thomson JA. 2008. NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. *Cell Stem Cell* 3:196–206.
- Yamashita A, Takada T, Yamamoto G, Torii R. 2006. Stable maintenance of monkey embryonic stem cells in the absence of bFGF. *Transplant Proc* 38:1614–1615.
- Yang W, Wei W, Shi C, Zhu J, Ying W, Shen Y, Ye X, Fang L, Duo S, Che J, Shen H, Ding S, Deng H. 2009. Pluripotin combined with leukemia inhibitory factor greatly promotes the derivation of embryonic stem cell lines from refractory strains. *Stem Cells* 27:383–389.
- Ying QL, Nichols J, Chambers I, Smith A. 2003. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* 115:281–292.
- Ying QL, Wray J, Nichols J, Battle-Morera L, Doble B, Woodgett J, Cohen P, Smith A. 2008. The ground state of embryonic stem cell self-renewal. *Nature* 453:519–523.
- Yoshida K, Chambers I, Nichols J, Smith A, Saito M, Yasukawa K, Shoyab M, Taga T, Kishimoto T. 1994. Maintenance of the pluripotential phenotype of embryonic stem cells through direct activation of gp130 signalling pathways. *Mech Dev* 45:163–171.
- Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. 2009. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 324:797–801.
- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. 2007a. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920.
- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. 2007b. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920 [Epub November 20, 2007].
- Yusa K, Rad R, Takeda J, Bradley A. 2009. Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. *Nat Methods* 6:363–369 [Epub March 31, 2009].
- Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Scholer HR, Duan L, Ding S. 2009. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 4:381–384 [Epub April 23, 2009].