Histol Histopathol (2011) 26: 643-650

**http://www.hh.um.es**

**Histology and Histopathology**

*Cellular and Molecular Biology*

## *Review*

# Induction of pluripotency in primordial germ cells

**Tohru Kimura and Toru Nakano**

Department of Pathology, Medical School, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka, Japan

**Summary.** Primordial germ cells (PGCs) are the founder cells of all gametes. PGCs differentiate from pluripotent epiblasts cells by mesodermal induction signals during gastrulation. Although PGCs are unipotent cells that eventually differentiate into only sperm or oocytes, they dedifferentitate to pluripotent stem cells known as embryonic germ cells (EGCs) *in vitro* and give rise to testicular teratomas *in vivo*, which indicates a "metastable" differentiation state of PGCs. We have shown that an appropriate level of phosphoinositide-3 kinase (PI3K)/Akt signaling, balanced by positive and negative regulators, ensures the establishment of the male germ lineage by preventing its dedifferentiation. Specifically, hyper-activation of the signal leads to testicular teratomas and enhances EGC derivation efficiency. In addition, PI3K/Akt signaling promotes PGC dedifferentiation via inhibition of the tumor suppressor p53, a downstream molecule of the PI3K/Akt signal. On the other hand, Akt activation during mesodermal differentiation of embryonic stem cells (ESCs) generates PGC-like pluripotent cells, a process presumably induced through equilibrium between mesodermal differentiation signals and dedifferentiation-inducing activity of Akt. The transfer of these cells to ESC culture conditions results in reversion to an ESC-like state. The interconversion between ESC and PGC-like cells helps us to understand the metastability of PGCs. The regulatory mechanisms of PGC dedifferentiation are discussed in comparison with those involved in the dedifferentiation of testicular stem cells, ESC pluripotency, and somatic nuclear reprogramming.

**Key words:** Phospohoinositide-3 kinase (PI3K), Akt, p53, Primordial germ cells (PGCs), Dedifferentiation, Embryonic germ (EG) cells, Teratoma, Pluripotency, Reprogramming

*Offprint requests to:* Dr. Tohru Kimura, Department of Pathology, Medical School, Graduate School of Frontier Biosciences, Osaka University, 2-2 Yamada-oka, Suita, Osaka, Japan 565.0871. e-mail: tkimura@patho.med.osaka-u.ac.jp or tnakano@patho.med.osaka-u.ac.jp

## Introduction

The germ lineage is a privileged cell lineage that can transmit genomic information to the next generation, thereby ensuring maintenance and evolution of the species (McLaren, 2003). Primordial germ cells (PGCs), which are the founder cells of all gametes, are specified from pluripotent epiblast cells in mammals (Fig. 1) (Saitou, 2009). PGCs emerge from a subset of epiblast cells that migrate to the extraembryonic region on embryonic day (E) 7 during early gastrulation in mice. PGC migration starts on E7.75 and PGCs colonize the genital ridges through the hindgut and dorsal mesentery by E11.5. After settlement onto the genital ridges, germ cells undergo a complex series of sex-dependent differentiation processes, i.e., spermatogenesis and oogenesis.

The expression of Blimp1, an essential transcription factor for PGC specification, commences in a few proximal epiblast cells at E6.25 (Ohinata et al., 2005; Vincent et al., 2005). Another transcription factor, Prdm14, which is also essential for PGC specification, is induced in PGC precursors slightly after the upregulation of Blimp1 (Yamaji et al., 2008). During specification to germ lineage cells, the gene expression patterns and epigenetic statuses of the cells are drastically altered by the cooperative actions of Blimp1 and Prdm14. Germline-specific genes, such as *PGC7*/*Stella*/*Dppa3* and *Dnd1*, are up-regulated in the nascent PGCs, i.e., the PGCs that have just emerged in the extraembryonic region at E6.75 to E7.0 (Kurimoto et al., 2008). In contrast, mesoderm-related genes are suppressed in the nascent PGCs, although they are transiently induced by the mesoderm-induction signals (Yabuta et al., 2006). Pluripotency-related genes, such as *Nanog* and *Sox2*, are re-activated in the nascent PGCs (Yabuta et al., 2006). PGCs undergo epigenetic reprogramming, which involves genome-wide alterations of repressive histone modification and DNA methylation patterns during the migration period of E7.75 to E9.5 (Seki et al., 2007). All of these changes

are presumably crucial for determining cell fate toward the PGCs. PGCs begin expressing gonocyte-markers, such as Mvh (mouse vasa homologue) and Mili, after arrival at gonads at E10.5-12.5.

Although PGCs are germline-committed cells, they can dedifferentiate into cells that have broader differentiation potential in mammals (Fig. 1) (Kimura et al., 2005). The PGCs isolated from E8.5-E12.5 embryos give rise to embryonic germ cells (EGCs) when cultured in the presence of the leukemia inhibitory factor (LIF), stem cell factor (SCF), and basic fibroblast growth factor (bFGF) (Fig. 2) (Matsui et al., 1992; Resnick et al., 1992). EGCs contribute to somatic and germ lineages after their introduction into blastocysts, whereas freshly isolated PGCs cannot perform these functions, indicating that the special culture conditions reprograms the PGCs to the pluripotent state (Labosky et al., 1994; Stewart et al., 1994; Durcova-Hills et al., 2008). In addition, testicular teratomas originate from PGCs, presumably through the dedifferentiation process (Stevens, 1967; Jiang and Nadeau. 2001). These observations suggest that the differentiation states of PGCs are "metastable" under specific circumstances.

In this review, we summarize our data regarding the roles of PI3K/Akt signaling and its downstream tumor suppressor p53 in the dedifferentiation of PGCs. We also discuss their function in other kinds of pluripotent stem cells including the dedifferentiation of testicular germline stem cells, regulation of embryonic stem cells (ESCs), and induction of pluripotency in somatic cell nuclei.

## PI3K/Akt signaling as a critical regulator of pluripotency in PGCs

### *PI3K/Akt signaling*

Phosphoinositide-3 kinase (PI3K)/Akt signaling is involved in diverse biological processes including proliferation, survival, migration, metabolism, tumorigenesis, and "stemness" (Brazil et al., 2004; Kimura and Nakano, 2009). PI3K/Akt signaling is activated by various growth factors, cytokines, chemokines, and adhesion molecules. PI3K produces phosphatidylinositol (3,4,5)-triphosphate (PtdIns (3,4,5)$P_3$) from PtdIns(4,5)$P_2$ (Cantley, 2002), and its function is counteracted by the antagonistic lipid phosphatase Pten (Stiles et al., 2004). PtdIns(3,4,5)$P_3$ transmits the signal via downstream effectors such as serine/threonine kinase Akt and GTPases Rac and Cdc42. Akt exerts its physiological and pathological effects by phosphorylation of several target proteins (Brazil et al., 2004).

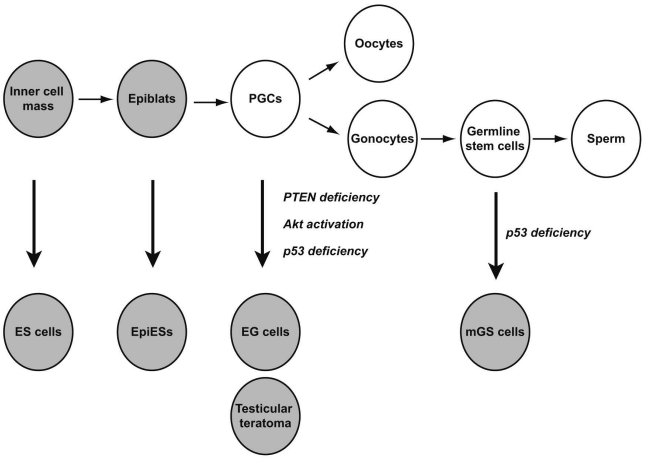


**Fig. 1.** Dedifferentiation of PGCs to pluripotent stem cells. PGCs differentiate from pluripotent epiblat cells during gastrulation. Although PGCs are committed to germ lineage, they give rise to pluripotent EGCs *in vitro* and testicular teratomas *in vivo*. Dedifferentiation of PGCs is promoted by the activation of PI3K/Akt signaling and inhibition of its downstream target p53. Germline stem cells in the testis also dedifferentiate to pluripotent stem cells. This process is enhanced by the inhibition of p53, but not by the activation of PI3K/Akt signaling. Activation of PI3K/Akt signaling and inhibition of p53 support self-renewal of ESCs and enhance the efficiency of somatic nuclear reprogramming. Pluripotent and germ-lineage cells are shown in gray and white, respectively (see text).

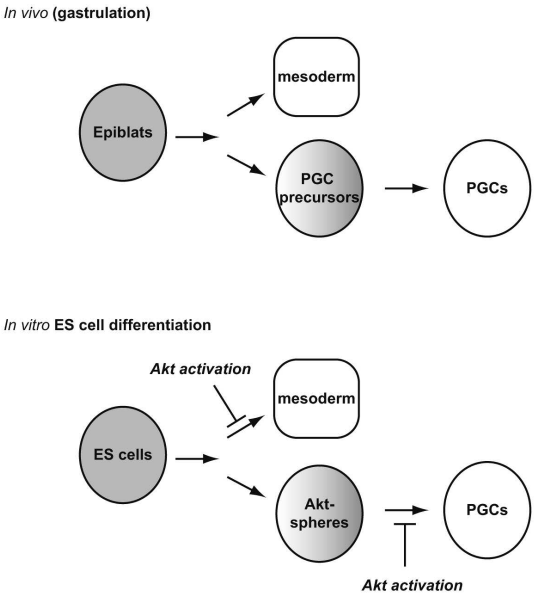


**Fig. 2.** Induction of PGC-like cells from ESCs. **Upper panel.** PGC precursors differentiate from pluripotent epiblast cells during gastrulation. Despite influences from mesodermal induction signals, mesoderm differentiation is inhibited, and the germline program is activated in the PGC precursors by the cooperative action of Blimp1 and Prdm14. **Bottom panel.** In the OP9 differentiation system, ESCs are induced for mesoderm by the signals emitted from OP9 feeder cells. However, the inhibitory effects of the Akt activation presumably suppressed the mesodermal differentiation, resulting in the generation of PGC-like cells known as Akt-sphere cells. The differentiation state of the Akt-spheres is maintained by the equilibrium between mesodermal differentiation signals and dedifferentiation-inducing activity of Akt.

### *PGC-specific* Pten*-deficient mice*

Our study of conditional *Pten*-deficient mice first demonstrated that enhancement of PI3K signaling promotes dedifferentiation of PGCs *in vivo* and *in vitro* (Kimura et al., 2003). The PGC-specific *Pten*-deficient mice were generated by using transgenic mice that expressed recombinase Cre from the *TNAP* (tissue-nonspecific alkaline phosphatase) locus. Expression of the gonadal PGC-marker Mvh was up-regulated in the PGCs of the E13.5 *Pten*-deficient embryos, as it was in littermate controls, suggesting that PGC differentiation normally proceeds to gonadal germ-cell stage in the absence of Pten. In contrast, a number of proliferative PGCs remained in the *Pten*-deficient male mice after E14.5, whereas male PGCs usually entered mitotic arrest at E13.5. At the same time, most *Pten*-deficient PGCs died by apoptosis, which led to germ-cell deficiency in newborn mutant males. However, a small number of germ cells survived to generate the teratomatous foci around E16.5. At birth, the fully developed testicular teratomas were observed in the mutant mice with hyper-phosphorylated Akt and lack of Mvh expression. Furthermore, the efficiency of EGC colony formation was enhanced in *Pten* mutants when E11.5 PGCs were cultured with LIF, SCF, and bFGF. These observations indicate that the appropriate signaling level regulated by the equilibrium between PI3K and Pten is a prerequisite for male germ-cell development and for prevention of PGC dedifferentiation.

### *Effects of conditional Akt activation on EGC derivation*

A series of experiments using the transgenic mice that expressed the myrAkt-Mer fusion protein showed that Akt is a critical downstream effector of PI3K with respect to the dedifferentiation of PGCs (Kimura et al., 2008). myrAkt-Mer is a fusion protein composed of the myristoylated, constitutive active form of Akt (myrAkt) and the ligand binding domain of the modified estrogen receptor (Mer). Although kinase activity of myrAkt-Mer is inactivated in the absence of the synthetic estrogen, 4-hydroxytamoxifen (4OHT), it is activated rapidly by the addition of 4OHT. Thus, myrAkt-Mer transgenic mice provide a valuable model system to examine the effects of transient Akt activation.

When PGCs of the myrAkt-Mer transgenic embryos were cultured in the presence of LIF, SCF, and bFGF, the derivation efficiency of EGCs was increased by the addition of 4OHT. The efficiency of EGC colony formation remained essentially the same as that of *Pten*-deficient PGCs. The enhanced EGC derivation by Akt activation was observed both in E8.5 migrating PGCs and in E11.5 gonadal PGCs. On the other hand, pharmacological inhibition of PI3K abolished EGC derivation, and this effect was completely rescued by Akt activation, demonstrating that activation of PI3K/Akt signaling is required for EGC derivation.

Treatment with bFGF is essential for EGC derivation because these cells do not derive from wild-type PGCs that are cultured with LIF and SCF, but without bFGF. In contrast, bFGF, but neither LIF nor SCF, induced Akt phosphorylation efficiently in the cultured PGCs. In the 4OHT-treated myrAkt-Mer transgenic PGCs, EGCs could be derived even in the absence of bFGF as efficiently as wild-type PGCs cultured with bFGF. In addition, transient Akt activation for 24 h from the beginning of the culture sufficiently induced the derivation of EGC lines. These observations show that the PI3K/Akt signaling axis downstream of bFGF mediates PGC dedifferentiation *in vitro*.

### *Tumor suppressor p53 as a downstream target of PI3K/Akt signaling*

We showed that the tumor suppressor p53 is a critical downstream target of Akt that promotes dedifferentiation of PGCs. Akt signaling suppressed the function of p53 in cultured PGCs due to Akt activation by 4OHT treatment in myrAkt-Mer PGCs. This, in turn, enhanced the stability and nuclear localization of Mdm2, which promotes degradation of p53, and suppressed p53 phosphorylation, which is required for its activation (Kimura et al., 2008). More importantly, the deletion of p53 recapitulated the effects of Akt activation in the EGC derivation. Namely, when cultured with LIF, SCF, and bFGF, efficiency of EGC derivation was enhanced in *p53*-deficient mice, and EGC lines could be established from *p53*-deficient mice without bFGF (Kimura et al., 2008). These results show that the inhibition of p53 on PI3K/Akt signaling contributes to the dedifferentiation of PGCs in culture. Considering that *p53*-deficient mice did not give rise to early onset of testicular teratomas with mixed genetic backgrounds (Harvey et al., 1993), it is highly likely that other critical downstream targets of Akt exist, particularly *in vivo*.

### *Roles of glycogen synthase kinase 3 (GSK3) and ß-catenin*

The transcriptional regulator ß-catenin is a candidate downstream target that mediates the PI3K/Akt signaling-induced PGC dedifferentiation, given that it is implicated in the regulation of various stem cells (Reya and Clevers, 2005). Activity of GSK3, which promotes ß-catenin degradation, is inhibited by Akt-mediated phosphorylation, resulting in the accumulation of ß-catenin in the nucleus (Moon et al., 2004). Inhibition of GSK3 or simultaneous inhibition of GSK3 and ERK (extracellular signal-regulated kinase) signal promotes ESC pluripotency and augments ESC derivation (Umehara et al., 2007; Ying et al., 2008).

GSK3 was hyper-phosphorylated, i.e., inactivated, in the PGCs by PI3K/Akt signaling activation (Kimura et al., 2008). However, it is unlikely that ß-catenin downstream of PI3K/Akt signaling promotes dedifferentiation of PGCs for the following reasons. First, *Pten*-deficient PGCs did not show nuclear

accumulation of ß-catenin (Kimura et al., 2006). Second, PGC-specific expression of nuclear-localized ß-catenin did not lead to testicular teratoma (Kimura et al., 2006). Third, the treatment of E11.5 PGCs with a GSK3 inhibitor, 6-bromoindirubin-3'-oxime (BIO), did not increase EGC derivation in the culture with LIF, SCF, and bFGF (Kimura et al., 2008). However, as dual inhibition using the MEK inhibitor PD0325901 and the GSK3 inhibitor CHIR99021 has been recently reported to promote EGC derivation in E8.5 PGCs (Leitch et al., 2010), further studies are necessary to elucidate the effect of GSK3 on EGC derivation.

## Metastablity in differentiation states of PGCs

### *Induction of PGC-like metastable pluripotency state by Akt activation*

Specification of PGCs takes place under the influence of mesodermal induction signals during gastrulation. To reproduce this condition *in vitro*, we combined the ESC differentiation system using OP9 feeder cells and Akt activation (Fig. 2) (Yamano et al., 2010). The OP9 differentiation system was used to induce mesodermal differentiation of ESCs, and the conditional activation of Akt signaling was used to inhibit ESC differentiation to three germ layers. The PGC-like spheres, referred to as Akt-sphere, could be induced from mouse ESCs and sustained for long periods under these culture conditions. The transcription factors Blimp1 and Prdm14, both of which are essential for PGC specification from epiblast cells, and their downstream PGC-specific genes were up-regulated in the Akt-sphere cells. However, when transplanted into testes of infertile mice, the sphere cells could not undergo spermatogenesis, but rather generated teratomas. Furthermore, the sphere cells reverted to ESC-like cells when transferred to ESC culture conditions. Thus, Akt-sphere cells are in a metastable and pluripotent state that is intermediate between ESCs and PGCs (Figs. 2, 3). The intermediate cells are presumably generated by the balance of antagonistic effects between mesoderm-inducing signals released by OP9 feeders and dedifferentiation-inducing activity of Akt signaling.

### *"Metastability" of differentiation states in PGCs*

According to the model recently proposed for cellular differentiation, the differentiation states are stabilized by intrinsic genetic networks and extracellular signalings (Graf and Stadtfeld, 2008; Enver et al., 2009; Hayashi and Surani, 2009a). As shown in Fig. 3, the differentiation state at the bottom of the "bowl," which is thermodynamically more stable than that at the "hilltop" in this model, is stabilized by intrinsic and external factors. One cell state can be "lifted-up" and "moved" to another cell state by exposure to the appropriate stimuli. For example, the mouse ESC-like pluripotent state is stabilized by the LIF signal, while the bFGF/activin signal stabilizes the epiblast stem cell (EpiSC)-like state. The ESC-like state is converted to the EpiSC-like state by culturing with bFGF/activin, whereas the EpiSC-like state is reverted to the ESC-like state by Klf4 expression and/or by inhibitors of the ERK and GSK3 cascades (black arrows).

The term 'metastability' was originally introduced to describe a spontaneous interconversion between ESC subpopulations, such as Nanog-positive and Nanog-negative cell populations (gray arrows) (Chambers et al., 2007; Hayashi et al., 2008). This concept has been extended to describe an interconversion between two differentiation states that is induced by culture conditions or by the introduction of specific genes, such as the interconversion between ESC-like and EpiSC-like states described above (black arrows in Fig. 3, left panel) (Guo et al., 2009; Hanna et al., 2009). In this regard, PGCs are in a "metastable" differentiation state, because PGCs are derived from the epiblast cells and can be reverted to the ESC-like state by exposure to bFGF, LIF,

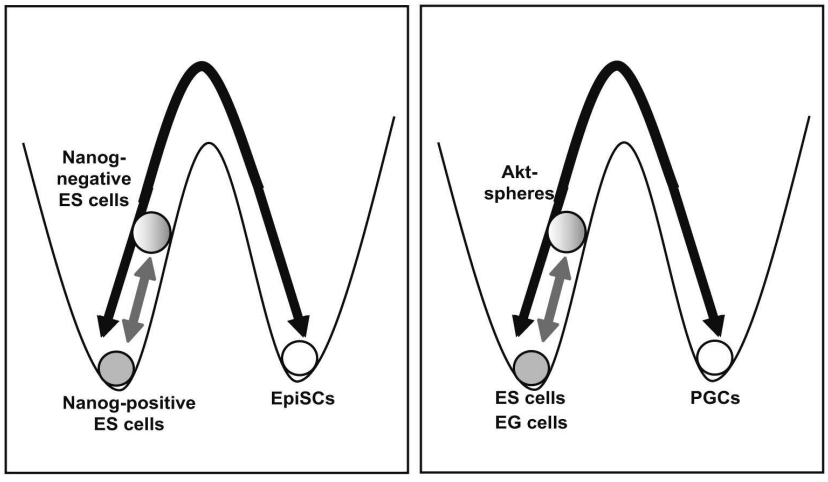


**Fig. 3.** Model summarizing the metastablity between pluripotent cells and PGCs. **Left and right** panels show the interconversion between ESCs and EpiSCs and that between pluripotent cells and PGCs, respectively. See the section "Metastability" of differentiation states in PGC in the text for details. In this figure, two distinct differentiation states are shown in gray and white.

and SCF (black arrows in Fig. 3, right panel) (Matsui et al., 1992; Resnick et al., 1992).

Both ESCs and Akt-sphere cells are in metastable equilibrium, considering their interconversion under distinct culture conditions (gray arrows in Fig. 3, right panel). Similarly, it has been reported that PGC-like cells spontaneously emerged from EpiSCs and can be reverted to EpiSCs under EpiSC culture conditions, suggesting an equilibrium between EpiSCs and PGC-like cells (Hayashi and Surani, 2009b). Thus, the PGC-like metastable differentiation states can be induced from pluripotent stem cells *in vitro*, which helps to elucidate the metastability between pluripotent cells and PGCs.

## Molecular mechanisms regulating pluripotency in testicular germline stem cells

Spermatogonial stem cells replenish their own population while supplying spermatocytes undergoing meiosis throughout life. Germline stem cells (GSCs), which are established from spermatogonia of neonatal mice, can be cultured for a long period of time in the presence of glial cell-line-derived neurotrophic factor (GDNF) (Kanatsu-Shinohara et al., 2003). After implantation to testes, GSCs differentiate to sperm, but do not develop teratomas, indicating that the unipotent differentiation ability of spermatogonia is retained in GSCs *in vitro*.

Pluripotent cells, which are called multipotent GS (mGS) cells, occasionally emerge in GSC culture (Kanatsu-Shinohara et al., 2004), although the efficiency is much lower than that of EGC formation from PGCs. Similar to ESCs and EGCs, mGS cells can be propagated in response to LIF, but not to GDNF, and differentiate to three germ layers and germ cells in chimeric mice. Similar pluripotent cell lines can also be derived from culture of adult mouse spermatogonial stem cells (Seandel et al., 2007; Ko et al., 2009).

GDNF is required for the self-renewal of GSCs *in vitro* and of spermatogonia *in vivo* (Meng et al., 2000; Kanatsu-Shinohara et al., 2003). PI3K inhibition prevented GSC proliferation in the presence of GDNF, and Akt activation supported self-renewal in the absence of GDNF (Lee et al., 2007). However, the derivation of mGS cells from GSCs was not promoted by Akt activation (Lee et al., 2007). These observations show that PI3K/Akt signaling supports self-renewing proliferation of GSCs, but does not promote their dedifferentiation. In contrast to PI3K/Akt signaling, the derivation efficiency of mGS cells was significantly enhanced in *p53*-deficient neonatal spermatogonia (Kanatsu-Shinohara et al., 2004).

## Regulation of the ESC pluripotency

### *Roles of PI3K/Akt signaling in ESC pluripotency*

Accumulating evidence has shown that PI3K/Akt signaling plays critical roles in self-renewing proliferation of ESCs. Whereas mouse ESCs self-renew in response to LIF, which activates signal transducers and the activator of transcription 3 (STAT3), primate ESCs respond to bFGF and activin for maintenance of pluripotency. PI3K/Akt signaling is activated by LIF and bFGF in mouse and primate ESCs, respectively (Paling et al., 2004). PI3K inhibition by the pharmacological inhibitor and dominant negative PI3K mutant decreased self-renewal of mouse ESCs in the presence of LIF (Paling et al., 2004). Furthermore, mouse ESCs expressing the constitutive active form of Akt maintained undifferentiated phenotypes in the absence of LIF (Watanabe et al., 2006). In addition, cynomolgous monkey ESCs carrying the *Akt-Mer* expression cassette self-renewed without feeder cells in the presence of 4OHT, but not in its absence (Watanabe et al., 2006). Therefore, activation of PI3K/Akt signaling is essential and sufficient for the self-renewing proliferation in both mouse and primate.

T cell leukemia 1 (Tcl1) enhances Akt kinase activity and mediates its nuclear translocation (Pekarsky et al., 2000). *Tcl1* is highly expressed in ESCs (Matoba et al., 2006), and its expression level is regulated by a zinc finger transcription factor Zfx and a histone demethylase for H3K9me2, Jmjd1a, both of which are required for self-renewal of ESCs (Galan-Caridad et al., 2007; Loh et al., 2007). Knockdown of *Tcl1* in mouse ESCs resulted in differentiation or reduced proliferation in the presence of LIF. This phenotype was restored by the expression of the constitutive active form of Akt (Ivanova et al., 2006; Matoba et al., 2006), suggesting that Tcl1 is required for Akt in self-renewing proliferation.

The mechanisms whereby PI3K/Akt signaling maintains ESC pluripotency remains to be elucidated. One candidate downstream target of PI3K/Akt signal is the pluripotent transcription factor Nanog, which plays a central role in the pluripotent transcriptional network with other transcriptional regulators such as Oct4, Sox2, Klf4, and c-myc. The expression of *Nanog* was downregulated by PI3K inhibition and was restored by inhibition of GSK3 with BIO (Takahashi et al., 2005; Storm et al., 2007), indicating that Akt-mediated GSK3 inhibition plays a role in *Nanog* expression. Additionally, the stability of c-myc, which is essential for ESC self-renewal (Cartwright et al., 2005), increased after Akt-mediated GSK3 inhibition (Bechard and Dalton, 2009).

### *Roles of p53 in ESC pluripotency*

It has been long known that the transcriptional activity and nuclear localization of p53 is suppressed in ESCs (Aladjem et al., 1998). The transactivation-deficient isoform of p53 called Δ40p53, which binds to the full-length p53 and sequesters it in the cytoplasm, is highly expressed in ESCs, but is down-regulated after differentiation induction (Ungewitter and Scrable, 2010). The inactivation and forced expression of the Δ40p53

isoform caused loss of pluripotency and inhibition of differentiation, respectively, in ESCs (Ungewitter and Scrable, 2010). As p53 represses pluripotency-related genes such as Nanog (Lin et al., 2005; Ungewitter and Scrable, 2010), inactivation of full-length p53 by Δ40p53 should be important for the maintenance of ESC pluripotency. In addition, down-regulation of Δ40p53 promotes differentiation of ESCs via activating full-length p53.

### *Induction of pluripotency in somatic cell nucleus*

The somatic cell nucleus can be reprogrammed to pluripotency by following three steps (Yamanaka and Blau, 2010). Somatic cells can re-acquire full developmental potency after being transferred into enucleated eggs. Pluripotency is also induced in somatic cell nuclei via hybridization with ESCs. The pluripotent stem cells, known as induced pluripotent stem (iPS) cells, can be induced by introducing transcription factors such as Oct-4, Sox2, Klf4, and c-myc that confer the ESC-like pluripotency transcriptional networks in somatic cell nuclei.

Conditional activation of Akt signaling in heterokaryons between ESCs and somatic cells enhanced derivation of ESC-like cells (Nakamura et al., 2008). In contrast, Akt activation in enucleated oocytes during somatic cell nuclear transfer (SCNT) resulted in developmental arrest at the 2- to 8-cell stages (Nakamura et al., 2008). Given that Akt activation in fertilized eggs promoted cell division and survival, but did not induce developmental arrest (Feng et al., 2007; Umehara, et al. 2007), it is likely that the developmental arrest in the Akt-activated clones was caused by the abrogated reprogramming during SCNT.

The suppression of p53 increased the efficiency of iPS cell generation in mouse and human (Zhao et al., 2008; Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Marion et al., 2009; Utikal et al., 2009). Activation of p53 and its downstream target p21 (encoded by Cdkn1a) pathway serves as a barrier for iPS cell generation. Thus, the molecules involved in PGC dedifferentiation play similar roles in the reprogramming of somatic nuclear reprogramming.

## Conclusion

In contrast to somatic cells, germ cells are reprogrammed to pluripotency only by cultivation under specific conditions. The expression of pluripotency transcription factors, such as Oct-4 and Sox2, in PGCs may facilitate efficient reprogramming of PGCs without genetic manipulation. The regulatory mechanisms of germ-cell dedifferentiation are largely conserved in the regulation of ESC pluripotency and of somatic cell reprogramming. Furthermore, the *in vitro* induction of the PGC-like pluripotent states provides a unique opportunity for understanding the metastability of pluripotency. Thus, studies on pluripotency induction in PGCs may elucidate the regulation of reprogramming to pluripotency and the metastability in germ and pluripotent cells.

*Acknowledgements.* This work was supported in part by grants from the Japan Society for the Promotion of Science, and the Ministry of Education, Culture, Sports, Science and Technology of Japan

## References

Aladjem M.I., Spike B.T., Rodewald L.W., Hope T.J., Klemm M., Jaenisch R. and Wahl G.M. (1998). ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. Curr. Biol. 8, 145-155.

Bechard M. and Dalton S. (2009). Subcellular localization of glycogen synthase kinase 3beta controls embryonic stem cell self-renewal. Mol. Cell. Biol. 29, 2092-2104.

Brazil D.P., Yang Z.Z. and Hemmings B.A. (2004). Advances in protein kinase B signalling: AKTion on multiple fronts. Trends Biochem. Sci. 29, 233-242.

Cantley L.C. (2002). The phosphoinositide 3-kinase pathway. Science 296, 1655-1657.

Cartwright P., McLean C., Sheppard A., Rivett D., Jones K. and Dalton S. (2005). LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Development 132, 885-896.

Chambers I., Silva J., Colby D., Nichols J., Nijmeijer B., Robertson M., Vrana J., Jones K., Grotewold L. and Smith A. (2007). Nanog safeguards pluripotency and mediates germline development. Nature 450, 1230-1234.

Durcova-Hills G., Tang F., Doody G., Tooze R. and Surani M.A. (2008). Reprogramming primordial germ cells into pluripotent stem cells. PLoS One 3, e3531.

Enver T., Pera M., Peterson C. and Andrews P.W. (2009). Stem cell states, fates, and the rules of attraction. Cell Stem Cell 4, 387-397.

Feng C., Yu A., Liu Y., Zhang J., Zong Z., Su W., Zhang Z., Yu D., Sun Q.Y. and Yu B. (2007). Involvement of protein kinase B/AKT in early development of mouse fertilized eggs. Biol. Reprod. 77, 560-568.

Galan-Caridad J.M., Harel S., Arenzana T.L., Hou Z.E., Doetsch F.K., Mirny L.A. and Reizis B. (2007). Zfx controls the self-renewal of embryonic and hematopoietic stem cells. Cell 129, 345-357.

Graf T. and Stadtfeld M. (2008). Heterogeneity of embryonic and adult stem cells. Cell Stem Cell 3, 480-483.

Guo G., Yang J., Nichols J., Hall J.S., Eyres I., Mansfield W. and Smith A. (2009). Klf4 reverts developmentally programmed restriction of ground state pluripotency. Development 136, 1063-1069.

Hanna J., Markoulaki S., Mitalipova M., Cheng A.W., Cassady J.P., Staerk J., Carey B.W., Lengner C.J., Foreman R., Love J., Gao Q., Kim J. and Jaenisch R. (2009). Metastable pluripotent states in NOD-mouse-derived ESCs. Cell Stem Cell 4, 513-524.

Harvey M., McArthur M.J., Montgomery C.A. Jr, Bradley A. and Donehower L.A. (1993). Genetic background alters the spectrum of tumors that develop in p53-deficient mice. FASEB J. 7, 938-943.

Hayashi K., Lopes S.M., Tang F. and Surani M.A. (2008). Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states. Cell Stem Cell 3, 391-401.

Hayashi K. and Surani M.A. (2009a). Resetting the epigenome beyond pluripotency in the germline. Cell Stem Cell 4, 493-498.

Hayashi K. and Surani M.A. (2009b). Self-renewing epiblast stem cells

exhibit continual delineation of germ cells with epigenetic reprogramming in vitro. Development 136, 3549-3556.

Hong H., Takahashi K., Ichisaka T., Aoi T., Kanagawa O., Nakagawa M., Okita K. and Yamanaka S. (2009). Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. Nature 460, 1132-1135.

Ivanova N., Dobrin R., Lu R., Kotenko I., Levorse J., DeCoste C., Schafer X., Lun Y. and Lemischka I.R. (2006). Dissecting self-renewal in stem cells with RNA interference. Nature 442, 533-538.

Jiang L.I. and Nadeau J.H. (2001). 129/Sv mice--a model system for studying germ cell biology and testicular cancer. Mamm. Genome 12, 89-94.

Kanatsu-Shinohara M., Inoue K., Lee J., Yoshimoto M., Ogonuki N., Miki H., Baba S., Kato T., Kazuki Y., Toyokuni S., Toyoshima M., Niwa O., Oshimura M., Heike T., Nakahata T., Ishino F., Ogura A. and Shinohara T. (2004). Generation of pluripotent stem cells from neonatal mouse testis. Cell 119, 1001-1012.

Kanatsu-Shinohara M., Ogonuki N., Inoue K., Miki H., Ogura A., Toyokuni S. and Shinohara T. (2003). Long-term proliferation in culture and germline transmission of mouse male germline stem cells. Biol. Reprod. 69, 612-616.

Kawamura T., Suzuki J., Wang Y.V., Menendez S., Morera L.B., Raya A., Wahl G.M. and Belmonte J.C. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. Nature 460, 1140-1144.

Kimura T. and Nakano T. (2009). Regulation of stem cell systems by PI3K/Akt signaling. In: Regulatory networks in stem cells. Rajasekhar V.K. and Vemuri M.C. (eds). Humana/Springer. New Jersey. pp 308-317.

Kimura T., Suzuki A., Fujita Y., Yomogida K., Lomeli H., Asada N., Ikeuchi M., Nagy A., Mak T.W. and Nakano T. (2003). Conditional loss of PTEN leads to testicular teratoma and enhances embryonic germ cell production. Development 130, 1691-1700.

Kimura T., Murayama K., Nakamura T., Watanabe S., Umehara H., Tomooka M. and Nakano T. (2005). Testicular teratomas: back to pluripotent stem cells. Prog. Mol. Subcell. Biol. 40, 133-150.

Kimura T., Nakamura T., Murayama K., Umehara H., Yamano N., Watanabe S., Taketo M.M. and Nakano T. (2006). The stabilization of beta-catenin leads to impaired primordial germ cell development via aberrant cell cycle progression. Dev. Biol. 300, 545-553.

Kimura T., Tomooka M., Yamano N., Murayama K., Matoba S., Umehara H., Kanai Y. and Nakano T. (2008). AKT signaling promotes derivation of embryonic germ cells from primordial germ cells. Development 135, 869-879.

Ko K., Tapia N., Wu G., Kim J.B., Bravo M.J., Sasse P., Glaser T., Ruau D., Han D.W., Greber B., Hausdorfer K., Sebastiano V., Stehling M., Fleischmann B.K., Brustle O., Zenke M. and Scholer H.R. (2009). Induction of pluripotency in adult unipotent germline stem cells. Cell Stem Cell 5, 87-96.

Kurimoto K., Yabuta Y., Ohinata Y., Shigeta M., Yamanaka K. and Saitou M. (2008). Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. Genes Dev. 22, 1617-1635.

Labosky P.A., Barlow D.P. and Hogan B.L. (1994). Embryonic germ cell lines and their derivation from mouse primordial germ cells. Ciba Found. Symp. 182, 157-168.

Lee J., Kanatsu-Shinohara M., Inoue K., Ogonuki N., Miki H., Toyokuni S., Kimura T., Nakano T., Ogura A. and Shinohara T. (2007). Akt mediates self-renewal division of mouse spermatogonial stem cells. Development 134, 1853-1859.

Leitch H.G., Blair K., Mansfield W., Ayetey H., Humphreys P., Nichols J., Surani M.A. and Smith A. (2010). Embryonic germ cells from mice and rats exhibit properties consistent with a generic pluripotent ground state. Development 137, 2279-2287.

Li H., Collado M., Villasante A., Strati K., Ortega S., Canamero M., Blasco M.A. and Serrano M. (2009). The Ink4/Arf locus is a barrier for iPS cell reprogramming. Nature 460, 1136-1139.

Lin T., Chao C., Saito S., Mazur S.J., Murphy M.E., Appella E. and Xu Y. (2005). p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. Nat. Cell Biol. 7, 165-171.

Loh Y.H., Zhang W., Chen X., George J. and Ng H.H. (2007). Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. Genes Dev. 21, 2545-2557.

Marion R.M., Strati K., Li H., Murga M., Blanco R., Ortega S., Fernandez-Capetillo O., Serrano M. and Blasco M.A. (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. Nature 460, 1149-1153.

Matoba R., Niwa H., Masui S., Ohtsuka S., Carter M.G., Sharov A.A. and Ko M.S. (2006). Dissecting Oct3/4-regulated gene networks in embryonic stem cells by expression profiling. PLoS One 1, e26.

Matsui Y., Zsebo K. and Hogan B.L. (1992). Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell 70, 841-847.

McLaren A. (2003). Primordial germ cells in the mouse. Dev. Biol. 262, 1-15.

Meng X., Lindahl M., Hyvonen M.E., Parvinen M., de Rooij D.G., Hess M.W., Raatikainen-Ahokas A., Sainio K., Rauvala H., Lakso M., Pichel J.G., Westphal H., Saarma M. and Sariola H. (2000). Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 287, 1489-1493.

Moon R.T., Kohn A.D., De Ferrari G.V. and Kaykas A. (2004) WNT and beta-catenin signalling: diseases and therapies. Nat. Rev. Genet. 5, 691-701.

Nakamura T., Inoue K., Ogawa S., Umehara H., Ogonuki N., Miki H., Kimura T., Ogura A. and Nakano T. (2008). Effects of Akt signaling on nuclear reprogramming. Genes Cells 13, 1269-1277.

Ohinata Y., Payer B., O'Carroll D., Ancelin K., Ono Y., Sano M., Barton S.C., Obukhanych T., Nussenzweig M., Tarakhovsky A., Saitou M. and Surani M.A. (2005). Blimp1 is a critical determinant of the germ cell lineage in mice. Nature 436, 207-213.

Paling N.R., Wheadon H., Bone H.K. and Welham M.J. (2004). Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. J. Biol. Chem. 279, 48063-48070.

Pekarsky Y., Koval A., Hallas C., Bichi R., Tresini M., Malstrom S., Russo G., Tsichlis P. and Croce C.M. (2000). Tcl1 enhances Akt kinase activity and mediates its nuclear translocation. Proc. Natl. Acad. Sci. USA 97, 3028-3033.

Resnick J.L., Bixler L.S., Cheng L. and Donovan P.J. (1992). Long-term proliferation of mouse primordial germ cells in culture. Nature 359, 550-551.

Reya T. and Clevers H. (2005). Wnt signalling in stem cells and cancer. Nature 434, 843-850.

Saitou M. (2009). Germ cell specification in mice. Curr. Opin. Genet. Dev. 19, 386-395.

Seandel M., James D., Shmelkov S.V., Falciatori I., Kim J., Chavala S., Scherr D.S., Zhang F., Torres R., Gale N.W., Yancopoulos G.D., Murphy A., Valenzuela D.M., Hobbs R.M., Pandolfi P.P. and Rafii S. (2007). Generation of functional multipotent adult stem cells from

GPR125+ germline progenitors. Nature 449, 346-350.

Seki Y., Yamaji M., Yabuta Y., Sano M., Shigeta M., Matsui Y., Saga Y., Tachibana M., Shinkai Y. and Saitou M. (2007). Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development 134, 2627-2638.

Stevens L.C. (1967). Origin of testicular teratomas from primordial germ cells in mice. J. Natl. Cancer Inst. 38, 549-552.

Stewart C.L., Gadi I. and Bhatt H. (1994). Stem cells from primordial germ cells can reenter the germ line. Dev. Biol. 161, 626-628.

Stiles B., Groszer M., Wang S., Jiao J. and Wu H. (2004). PTENless means more. Dev. Biol. 273, 175-184.

Storm M.P., Bone H.K., Beck C.G., Bourillot P.Y., Schreiber V., Damiano T., Nelson A., Savatier P. and Welham M.J. (2007). Regulation of Nanog expression by phosphoinositide 3-kinase-dependent signaling in murine embryonic stem cells. J. Biol. Chem. 282, 6265-6273.

Takahashi K., Murakami M. and Yamanaka S. (2005). Role of the phosphoinositide 3-kinase pathway in mouse embryonic stem (ES) cells. Biochem. Soc. Trans. 33, 1522-1525.

Umehara H., Kimura T., Ohtsuka S., Nakamura T., Kitajima K., Ikawa M., Okabe M., Niwa H. and Nakano T. (2007). Efficient derivation of embryonic stem cells by inhibition of glycogen synthase kinase-3. Stem Cells 25, 2705-2711.

Ungewitter E. and Scrable H. (2010). Delta40p53 controls the switch from pluripotency to differentiation by regulating IGF signaling in ESCs. Genes Dev. 24, 2408-2419.

Utikal J., Polo J.M., Stadtfeld M., Maherali N., Kulalert W., Walsh R.M., Khalil A., Rheinwald J.G. and Hochedlinger K. (2009). Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. Nature 460, 1145-1148.

Vincent S.D., Dunn N.R., Sciammas R., Shapiro-Shalef M., Davis M.M., Calame K., Bikoff E.K. and Robertson E.J. (2005). The zinc finger transcriptional repressor Blimp1/Prdm1 is dispensable for early axis formation but is required for specification of primordial germ cells in the mouse. Development 132, 1315-1325.

Watanabe S., Umehara H., Murayama K., Okabe M., Kimura T. and Nakano T. (2006). Activation of Akt signaling is sufficient to maintain pluripotency in mouse and primate embryonic stem cells. Oncogene 25, 2697-2707.

Yabuta Y., Kurimoto K., Ohinata Y., Seki Y. and Saitou M. (2006). Gene expression dynamics during germline specification in mice identified by quantitative single-cell gene expression profiling. Biol. Reprod. 75, 705-716.

Yamaji M., Seki Y., Kurimoto K., Yabuta Y., Yuasa M., Shigeta M., Yamanaka K., Ohinata Y. and Saitou M. (2008). Critical function of Prdm14 for the establishment of the germ cell lineage in mice. Nat. Genet. 40, 1016-1022.

Yamanaka S. and Blau H.M. (2010). Nuclear reprogramming to a pluripotent state by three approaches. Nature 465, 704-712.

Yamano N., Kimura T., Watanabe-Kushima S., Shinohara T. and Nakano T. (2010). Metastable primordial germ cell-like state induced from mouse embryonic stem cells by Akt activation. Biochem. Biophys. Res. Commun. 392, 311-316.

Ying Q.L., Wray J., Nichols J., Batlle-Morera L., Doble B., Woodgett J., Cohen P. and Smith A. (2008). The ground state of embryonic stem cell self-renewal. Nature 453, 519-523.

Zhao Y., Yin X., Qin H., Zhu F., Liu H., Yang W., Zhang Q., Xiang C., Hou P., Song Z., Liu Y., Yong J., Zhang P., Cai J., Liu M., Li H., Li Y., Qu X., Cui K., Zhang W., Xiang T., Wu Y., Liu C., Yu C., Yuan K., Lou J., Ding M. and Deng H. (2008). Two supporting factors greatly improve the efficiency of human iPSC generation. Cell Stem Cell 3, 475-479.

Accepted February 11, 2011