

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Molecular Mechanisms of Embryonic Stem Cell Pluripotency

Guofeng Han, Hongtao Wang and Jijun Hao

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54365>

1. Introduction

Embryonic stem (ES) cells isolated from the inner cell mass (ICM) of blastocysts possess the defining pluripotency: unlimited self-renewal and giving rise to all cells of the organism[1, 2]. Thus, ES cells hold great promise for regenerative medicine to treat many diseases including heart failure, diabetes, Alzheimer's and Parkinson's disease by replacing the damaged cells with ES cell-derived healthy ones. The recent advent of induced pluripotent stem (iPS) cells reprogrammed from somatic cells has the potential to revolutionize the field of regenerative medicine since patient-derived iPS cells, in principle, circumvent the ethical problems and immune rejection associated with human ES cells[3]. Nevertheless, the future clinical translation of ES cells and iPS cells is facing numerous hurdles. Understanding the molecular mechanisms that impart ES cells with pluripotency may help address some of these challenges. The past few years have seen tremendous progress in understanding of mechanisms which govern ES cell pluripotency. In this chapter, we will review critical signaling and transcription factor networks that have been identified to maintain ES cell pluripotency.

2. Signaling pathways of ES cells

ES cells require extrinsic growth factors to maintain their pluripotency in culture. These extrinsic growth factors act on different signaling pathways to regulate intrinsic transcription factor networks to sustain ES cells in the undifferentiated state. The signaling pathways required to support pluripotency in mouse ES cell are distinct from those in human ES cells (Figure 1).

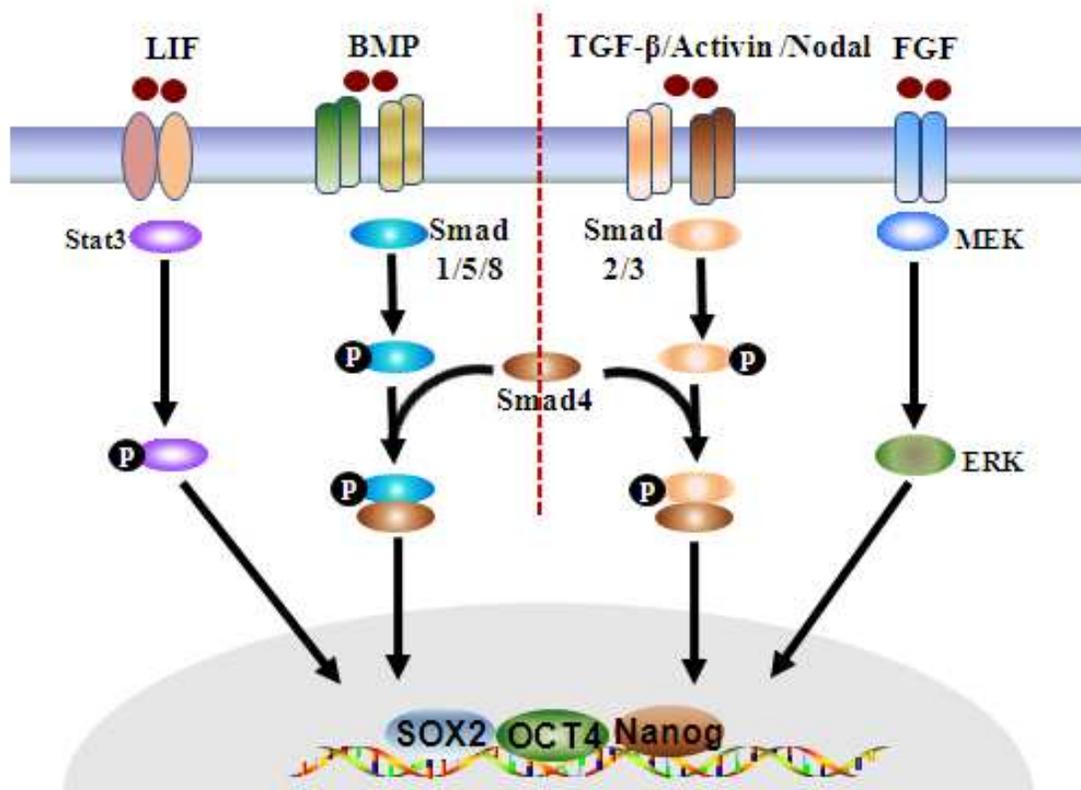


Figure 1. Exogenous growth factors signal through distinct signaling pathways to regulate transcription factors for ES cell pluripotency.

2.1. LIF/JAK/STAT3 pathway

Mouse ES cells were originally cultured on feeder layers derived from mouse embryonic fibroblasts (MEF). Later it was found that Leukaemia Inhibitory Factor (LIF), a member of the Interleukin-6 cytokines produced by MEFs, was the key factor to maintain pluripotency of mouse ES cells by inhibiting their differentiation[4]. Upon LIF binding, the LIF receptor recruits gp130 to form a heterodimer which subsequently activates Janus kinase (JAK) through transphosphorylation[5]. Activated JAK then phosphorylates gp130, creating a docking site to bind the SH2 domain of Signal Transducers and Activators of Transcription 3 (STAT3)[6-9]. Once STAT3 binds to the gp130 docking site, JAK then phosphorylates the recruited STAT3. Phosphorylated STAT3 forms a homodimer, which subsequently translocate into the nucleus, where it binds to gene enhancers to regulate target gene expression[10-12].

Although the LIF/JAK/STAT3 pathway has been well documented to maintain pluripotency of mouse ES cells in the presence of serum, the mechanisms by which activated STAT3 functions in this regard is poorly understood. Recently, studies in identification of STAT3 target genes have improved our understanding of activated STAT3 in maintaining pluripotency. Chen et al identified 718 STAT3-bound genomic sites that were co-occupied by pluripotency transcription markers (Oct4, Sox2 and Nanog) by using chromatin immunoprecipitation sequencing (ChIP-seq)[12]. In addition, Kidder and colleagues found that STAT3 target genes

enriched in ES cells were downregulated in differentiated cells by mapping STAT3 binding targets in mouse ES cells and differentiated embryoid bodies (EBs)[13]. Along with these results, it has been demonstrated that knocking down STAT3-target genes induces activation of endodermal and mesodermal genes, supporting the conclusion that STAT3 prevents mESC differentiation by suppressing lineage-specific genes[14].

Interestingly, the LIF receptor and gp130 are also expressed in human ES cells and human LIF can induce STAT3 phosphorylation and nuclear translocation in human ES cells. However, human LIF is unable to maintain the pluripotent state of human ESs, suggesting that mouse and human ES cells require distinct signaling mechanisms to govern their pluripotency[15].

2.2. TGF- β signaling

TGF- β superfamily consists of more than 40 members, including TGF- β , Activin, Nodal, and bone morphogenetic proteins (BMPs). The TGF- β members transduce signals by binding to heteromeric complexes of serine/threonine kinase receptors, type I and type II receptors, which subsequently activate intracellular Smad proteins. Smads 2 and 3 are specifically activated by activin, nodal and TGF- β ligands, whereas Smads 1, 5 and 8 are activated by BMP ligands[16, 17] (Figure 1). The TGF- β -related signaling pathways play complex roles in regulating the pluripotency and cell fate of ES cells.

2.2.1. BMP signaling pathway

Bone Morphogenetic Protein (BMP) is a subset of the TGF- β superfamily[18]. When BMP ligands bind to type II BMP receptors (BMPRII), BMPRII then recruits and phosphorylates type I BMP receptors (BMPRI). Activated type I receptors subsequently phosphorylate BMP-responsive SMAD1/5/8 which then forms a complex with SMAD4 and translocates into nucleus to regulate target gene expression (Figure 1). In mouse ES cells, LIF can substitute MEF feeder layers in maintaining pluripotency in the presence of animal serum by activating the transcription factor STAT3. However, in serum-free cultures, LIF is insufficient to block neural differentiation and maintain pluripotency. Recently, Ying et al reported that BMP was able to replace serum to maintain pluripotency of mouse ES cells in the presence of LIF. BMP has been shown to phosphorylate SMAD1/5 and activate inhibitors of differentiation (*Id*) genes, which block neural differentiation by antagonizing neurogenic transcription factors[19]. In the absence of MEF and serum, exogenous LIF, in combination with BMP4 proteins, can sufficiently maintain the pluripotency of mouse ES cells derived from "permissive" mouse strains.

In contrast to a maintenance role in mouse ES cell pluripotency, BMP has been shown to promote human ES cells differentiation to trophoblasts, and inhibiting BMP signaling with the BMP antagonist, Noggin, sustains the undifferentiated state of human ES cells[20, 21]. In consistence, dorsomorphin and DMH1, small molecule BMP inhibitors previously identified in our lab, were shown to promote long-term self-renewal an pluripotency of human ES cells, presumably by inhibiting BMP induced extraembryonic lineage differentiation[22-25].

2.2.2. *TGF- β /activin/nodal signaling pathway*

Although MEFs feeder layers were initially used to co-culture both mouse and human ES cells, signal factors secreted from MEFs to maintain pluripotency of the two types of ES cells are fundamentally different. Sato et al first discovered that TGF- β and Nodal genes were highly expressed in undifferentiated human ES cells[26]. Beattie et al later reported that Activin A, a member of the TGF- β superfamily, was secreted by MEFs, and medium enriched with activin A can replace MEF feeder-layers or MEF-conditioned media to maintain human ES cells in an undifferentiated state[27]. In consistence, James et al demonstrated that the TGF- β /Activin/Nodal pathway was activated through the transcription factors Smad2/3 in undifferentiated human ES cells[28]. The notion that TGF- β /Activin/Nodal signaling supports human ES self-renewal and pluripotency is further supported by the fact that recombinant Activin or Nodal stimulation induces higher levels of pluripotent protein expression (Oct4 and Nanog), while inhibition of TGF- β /Activin/Nodal signaling with Lefty or Follistatin decreases expression of these pluripotent proteins in human ES cells[29, 30].

Recent studies have focused on understanding the molecular mechanisms of TGF- β /Activin/Nodal signaling in retaining human ES cells pluripotency. Xu and colleagues showed that TGF- β /Activin/Nodal signaling activated Smad2/3 which subsequently binds to the Nanog promoter in undifferentiated human ES cells to induce expression of Nanog, a pluripotent transcription factor[31]. Additionally, mutating the putative Smad-binding sites reduced the response of Nanog to modulation of TGF- β signaling[31]. Nanog was also shown to coordinate with Smad2 in a negative-feedback loop to inhibit human ES cell differentiation[32]. In contrast to its important role in maintaining human ES cell pluripotency, the TGF- β /Activin/Nodal signaling is not essential for pluripotency of mouse ES cells. Although this pathway was shown to be active in undifferentiated mouse ES cells as assessed by phosphorylation of smad 2/3, inhibition of smad 2/3 phosphorylation by SB431542 had no effect on the undifferentiated state of mouse ES cells[28]. However, the TGF- β /Activin/Nodal signaling may play a role in mouse ES proliferation. A recent study showed that Inhibition of TGF- β /Activin/Nodal signaling by Smad7 or SB-431542 dramatically decreased mouse ES cell proliferation without effect on their pluripotency[33].

2.2.3. *Growth and Differentiation factor 3 (GDF-3)*

GDF-3 is another TGF-beta superfamily member that plays opposite roles in mouse and human ES cells. GDF-3, which acts as a BMP antagonist by direct binding to BMP-4, is specifically expressed in the pluripotent state of both mouse and human ES cells[34]. Ectopic expression of GDF-3 leads to the maintenance of pluripotency in human ES cells, whereas a similar effect is observed in mouse ES cells when GDF-3 levels are decreased. In the absence of LIF, GDF-3-deficient mouse ES cells can still sustain pluripotent markers[34]. These results are consistent with previously discussed BMP signals which can promote pluripotency of mouse ES cells, but cause differentiation of human ES cells. Thus lower concentrations of BMP antagonists, such as GDF-3, may enhance pluripotency in mouse ES cells, whereas higher levels of GDF-3 may favor pluripotency of human ES cells by abrogating BMP signaling.

2.3. FGF/MEK signaling

The importance of Fibroblast growth factor (FGF) signaling for human ES cells pluripotency is highlighted by the facts that human ES cells are traditionally cultured in the presence of Fibroblast growth factors (FGFs) either on fibroblast feeder layers or in fibroblast-conditioned medium[35, 36]. Studies have demonstrated that all four FGF receptors (FGFR1, FGFR3 and FGFR4) and several components (SOS1, PTPN11 and RAF1) of their downstream activation cascade are significantly upregulated in undifferentiated human ES cells, in comparison to differentiated human ES cells[37-39]. In consistence, withdrawal of FGFs or inhibition of FGF signaling by a FGFR inhibitor, SU5402, rapidly induces human ES cell differentiation[40-42].

Although the pluripotency maintenance role of exogenous FGFs in human ES cell has been known for a long time, the molecular mechanisms by which they function remain unclear. FGFs signal by binding to FGF receptors (FGFRs), and activate multiple signaling cascades, including Mitogen-Activated Protein Kinases (MAPKs), the Janus kinase/signal transducer and activator of transcription (Jak/Stat), phosphatidylinositol 3-kinase (PI3K) and phosphoinositide phospholipase C (PLC γ) pathway[43]. Several studies have highlighted the FGF contribution to the maintenance of human ES cells mainly through the FGF/MEK pathway (Figure 1), [44, 45]. Studies have showed that FGF2 induces feeder layer cells to secrete TGF β 1 and insulin-like growth factor 2 (IGF2), which can subsequently promote the undifferentiated state of human ES cells[46, 47]. Bendall et al further reported that the function of exogenous FGFs in promoting ES self-renewal could be replaced by addition of IGF2 alone, suggesting an indirect role of FGFs for human ES cell growth. However, this model was challenged in subsequent publications from Wang et al who reported that exogenous IGF2 alone was insufficient to maintain undifferentiated growth of human ES cells, and they proposed that FGFs may play a direct role in blocking caspase-activated apoptosis through anoikis in human ES cells[48]. Recently, Eiselleova and colleagues postulated a new model whereby endogenous FGF-2 signaling maintained the undifferentiated state and survival of human ESCs, while exogenous FGF-2 mainly suppress cell death and apoptosis genes, thus indirectly contributing to the maintenance of human ES cell pluripotency[49].

FGF signaling in mouse ES cells has also been extensively investigated. Mouse ES cells genetically deficient in Fgf4 and extracellular-signal regulated kinase 2 (Erk2) differentiate inefficiently. These results can be reproduced using inhibitors of FGF receptor and ERK, suggesting blockage of the FGF/MEK signaling pathway promotes mouse ES cell pluripotency[50-52]. Indeed, serum-free mouse ES cell medium supplemented with FGF/MEK inhibitors and LIF permits the derivation of mouse ES cells in the absence of feeders from strains normally considered non-permissive[53]. In addition, a recently identified compound, Pluripotin/SC1, has been shown to maintain mouse ES pluripotency by inhibiting ERK1 and activating the phosphoinositide-3 kinase (PI3K) pathway through blocking RasGAP[54-56] [57, 58]. Although inhibition of FGF/MEK pathway can attenuate ES cell differentiation, it is insufficient to support mouse ES cell self-renewal. Combination of the MEK inhibitor PD0325901 with the Glycogen synthase kinase-3 (GSK-3) inhibitor CHIR99021 (known as 2i) can efficiently sustain the pluripotency of mouse ES cells in the absence of exogenous cytokines[59, 60]. Several groups dem-

onstrated that improvement of mouse ES cell pluripotency by inhibition of GSK-3 occurred via Wnt/ β -catenin signaling, whereas many others argued that GSK3 was likely to exert β -catenin independent effects in ES cells[59, 61-67].

As demonstrated above, human and mouse ES cells are both derived from blastocyst-stage embryos, but they require different biological signals for maintaining pluripotency. In general, mouse ES cells maintain their pluripotency by activating LIF/STAT3 and BMP signaling, while human ES cells require TGF- β /Nodal and FGF/MEK pathways. Interestingly, several pathways, such as BMP and FGF/MEK, have completely opposing effects on maintaining the pluripotency of mouse and human ES cells. Activation of BMP signaling and inhibition of the FGF/MEK pathway promote mouse ES self-renewal, whereas inhibition of BMP signaling and activation of FGF/MEK pathway sustain human ES cell pluripotency. These distinct signaling effects on pluripotency may reflect intrinsic differences between mouse and human ES cells. Recent studies have demonstrated that conventional human ES cells do not represent the “ground or naïve state” of stemness, but rather a more developmentally mature “primed state” resembling mouse epiblast stem cells (mEpiSCs) found in the post-implantation, pre-gastrulation stage of embryos [68-74]. Conventional human ES cells exhibit numerous similarities to the mouse EpiSCs over mouse ES cells (Table 1). For instance, conventional human ES cells and mouse EpiSCs display flattened cell colonies and epigenetic X-chromosome inactivation (XiXa), and require Activin and FGF for pluripotency maintenance. In contrast, mouse ES cells exhibit dome-shaped colony morphology and epigenetic activation of both X-chromosome (XaXa), and require LIF/STAT3 signaling to promote self-renewal. Subsequent studies have demonstrated that the medium containing “2i” (MEK inhibitor and GSK-3 inhibitor), when supplemented with other factors (such as forskolin), can efficiently convert conventional human ES cells into a ground or “naïve” state with display of hallmark features of mouse ES cells. This medium can also maintain human ES cell pluripotency at the naïve state [69, 70, 72, 75-78].

property	mESCs	mEpiSCs	hESCs	hiPSCs
morphology	domed	flattened	flattened	flattened
clonogenicity	high (single cells)	low (clumps)	low (clumps)	low (clumps)
response to LIF/Stat3	self-renewal	none	none	none
response to Activin/bFGF	differentiation	self-renewal	self-renewal	self-renewal
response to BMP	self-renewal	differentiation	differentiation	differentiation
XX status	XaXa	XaXi	XaXi	XaXi
teratoma	yes	yes	yes	yes
chimaera	yes	no	ND	ND

Table 1. Comparison of the properties of mouse ES cells (mESCs), mouse epiblast stem cells (mEpiSCs), human ES cells (hESCs) and human iPS cells (hiPSCs).

3. The regulatory network of pluripotency factors

ES cell pluripotency is conferred by a unique transcriptional network[79]. Early global transcriptional profiles and genetic studies have identified several critical transcription factors that are required for the pluripotency of ES cells, such as Oct4, Sox2, Nanog, Foxd3 and Id, etc [80-88]. Here we will mainly focus on Oct4, Sox2 and Nanog, three key transcription factors of the core pluripotency transcriptional network.

3.1. OCT4 and SOX2

OCT4 (also known as Oct3), a POU domain-containing transcription factor, was one of the first transcription factors identified as essential for both early embryo development and pluripotency maintenance in ES cells[84, 89]. The expression of Oct4 is activated at the 8-cell stage and is later restricted to the inner cell mass (ICM) and germ cells in early mouse embryogenesis *in vivo* [89-92]. Oct4 is highly expressed in both human and mouse ES cells, and its expression diminishes when these cells differentiate and lose pluripotency. Oct4 regulates a broad range of target genes including *Fgf4*, *Utf1*, *Opn*, *Rex1*/*Zfp42*, *Fbx15*, *Sox2* and *Cdx2*[93-95]. Repression of Oct4 activity in ES cells upregulates *Cdx2* expression, leading to ES cell differentiation into trophoblast[96]. Oct4 is also known to activate downstream genes by binding to enhancers carrying the octamer-sox motif (Oct-Sox enhancer), for synergistic activation with Sox2. In contrast with its target genes, little is known about Oct4 upstream regulators. The Oct4 promoter contains conserved distal and proximal enhancers that can either repress or activate its expression depending on the binding factors occupying these sites[97, 98]. The precise level of Oct4 is important for ES cell fate determination. Loss of Oct4 causes inappropriate differentiation of ES cells into trophoblast, whereas overexpression of Oct4 results in differentiation into primitive endoderm and mesoderm[99, 100].

Sox2 is an HMG-box transcription factor that is detected in pluripotent cell lineages and the nervous system[101-103]. Inactivate Sox2 *in vivo* results in early embryonic lethality due to the failure of ICM maintenance[102]. Sox2 can form a complex with the Oct4 protein to occupy Oct-Sox enhancers to regulate target gene expression. Oct-Sox enhancers are found in the regulatory region of most of the genes that are specifically expressed in pluripotent stem cells, such as Oct4, Sox2, Nanog, *Utf1*, *Lefty*, *Fgf4* and *Fbx15*[93, 94, 104-108].

3.2. Nanog

Nanog is another homeobox-containing transcription factor that is specifically expressed in pluripotent ES cells. The essential role of Nanog in maintaining the pluripotency of ES cells is highlighted by the facts that Nanog-deficient ES cells are prone to differentiation, whereas forced expression of Nanog partially renders ES cells self-renewal potential in the absence of LIF[85, 86, 109]. How Nanog regulates stem cell pluripotency remains entirely unknown. Studies have indicated that Nanog may maintain ES cell pluripotency by 1) downregulating downstream genes essential for cell differentiation such as *Gata4* and *Gata6* and 2) activating the expression of genes necessary for self-renewal such as *Rex1* and *Id*[19, 85, 86]. Although it is widely accepted that Nanog, like Oct4 and Sox2, play a central role in

pluripotency maintenance, this dogma has been challenged by a subsequent report that Nanog protein levels are undetectable in a fraction of ES cells that express Oct4, and the pure populations of Nanog^{-/-} ES cells can be propagated without losing expression of other pluripotency markers[110].

Little is known about the mechanism by which Nanog is regulated in ES cells. Recently, Suzuki et al showed that Nanog expression was upregulated by BrachyuryT and STAT3 in mouse ES cells[111]. In human ES cells and in mouse EpiSCs, Vallier et al reported that Activin/Nodal signaling stimulated expression of Nanog, which in turn prevents FGF-induced neuroectoderm differentiation [112]. In addition, several studies indicated that the Oct4/Sox2 complex was directly bound to the Nanog promoter to regulate target gene expression [106, 107, 113]. Genomic studies have revealed that Oct4, Sox2, and Nanog frequently bind the same regulatory regions in undifferentiated mouse and human ESCs, and that these binding sites are often in close proximity to one another[113-116]. These results indicate that Oct4, Sox2, and Nanog may physically interact with each other and coordinately regulate target genes in some cases. Additionally, Goke and colleagues reported that combinatorial binding sites of the Oct4/Sox2/Nanog were more conserved between mouse and human ES cells than individual binding sites were [113, 114, 117-119].

4. Summary

Understanding the molecular mechanism of pluripotency can greatly expand our knowledge of ES cell biology and facilitate future stem cell clinical applications. In the past few years, we have seen tremendous advances in understanding ES cell pluripotency. Although mouse ES cells and conventional human ES cells require distinct signaling pathways to maintain pluripotency, they display similar gene expression profiles, activities of transcription factors (such as Oct4, Nanog and Sox2) and transcription factor networks. Our understanding of pluripotency has been further expanded by the advent of iPS cells and the very recent discovery that conventional human ES cells are more equivalent to mouse EpiSCs, but rather “naïve state” of mouse ES cells. Nevertheless, our knowledge of the molecular mechanisms of ES cell pluripotency is still very limited. For instance, it remains unknown how growth factors establish and control transcriptional networks to regulate pluripotency and how ES cells respond so precisely to exogenous cues. Given the rapid advance in ES cell biology, we anticipate the molecular mechanisms underlying pluripotency of ES cells will soon be uncovered and pluripotent stem cells, such as ES cells and iPS cells, will be widely used for clinical applications in the near future.

Acknowledgements

This work is funded by the seed fund of the Western University of Health Sciences.

Author details

Guofeng Han¹, Hongtao Wang² and Jijun Hao^{3*}

*Address all correspondence to: jhao@westernu.edu

1 Department of Nephrology, Shanghai Jimin Hospital, Shanghai, China

2 Department of Burn and Cutaneous Surgery, XiJing Hospital, the Fourth Military Medical University, Xi'an, China

3 College of Veterinary Medicine, Western University of Health Sciences, Western University of Health Sciences, Pomona, CA, USA

References

- [1] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981;292(5819):154-6.
- [2] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282(5391):1145-7.
- [3] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663-76.
- [4] Smith AG, Heath JK, Donaldson DD, Wong GG, Moreau J, Stahl M, et al. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature*. 1988;336(6200):688-90.
- [5] Niwa H, Burdon T, Chambers I, Smith A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. *Genes Dev*. 1998;12(13):2048-60.
- [6] Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE, Jr., Yancopoulos GD. Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science*. 1995;267(5202):1349-53.
- [7] Hemmann U, Gerhartz C, Heesel B, Sasse J, Kurapkat G, Grotzinger J, et al. Differential activation of acute phase response factor/STAT3 and Stat1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. II. Src homology SH2 domains define the specificity of stat factor activation. *J Biol Chem*. 1996;271(22):12999-3007.
- [8] Gerhartz C, Heesel B, Sasse J, Hemmann U, Landgraf C, Schneider-Mergener J, et al. Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. I. Definition of a novel phosphotyrosine motif mediating STAT1 activation. *J Biol Chem*. 1996;271(22):12991-8.

- [9] Ihle JN, Kerr IM. Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet.* 1995;11(2):69-74.
- [10] Auernhammer CJ, Melmed S. Leukemia-inhibitory factor-neuroimmune modulator of endocrine function. *Endocr Rev.* 2000;21(3):313-45.
- [11] Reich NC, Liu L. Tracking STAT nuclear traffic. *Nat Rev Immunol.* 2006;6(8):602-12.
- [12] Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell.* 2008;133(6):1106-17.
- [13] Kidder BL, Yang J, Palmer S. STAT3 and c-Myc genome-wide promoter occupancy in embryonic stem cells. *PLoS One.* 2008;3(12):e3932.
- [14] Bourillot PY, Aksoy I, Schreiber V, Wianny F, Schulz H, Hummel O, et al. Novel STAT3 target genes exert distinct roles in the inhibition of mesoderm and endoderm differentiation in cooperation with Nanog. *Stem Cells.* 2009;27(8):1760-71.
- [15] Daheron L, Opitz SL, Zaehres H, Lensch MW, Andrews PW, Itskovitz-Eldor J, et al. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. *Stem Cells.* 2004;22(5):770-8.
- [16] Wrana JL, Attisano L, Carcamo J, Zentella A, Doody J, Laiho M, et al. Tgf-Beta Signals through a Heteromeric Protein-Kinase Receptor Complex. *Cell.* 1992;71(6):1003-14.
- [17] Schmierer B, Hill CS. TGF beta-SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Bio.* 2007;8(12):970-82.
- [18] Sebald W, Nickel J, Zhang JL, Mueller TD. Molecular recognition in bone morphogenetic protein (BMP)/receptor interaction. *Biol Chem.* 2004;385(8):697-710.
- [19] Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell.* 2003;115(3):281-92.
- [20] Xu RH, Chen X, Li DS, Li R, Addicks GC, Glennon C, et al. BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nat Biotechnol.* 2002;20(12):1261-4.
- [21] Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat Methods.* 2005;2(3):185-90.
- [22] Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol.* 2008;4(1):33-41.

- [23] Hao J, Ho JN, Lewis JA, Karim KA, Daniels RN, Gentry PR, et al. In vivo structure-activity relationship study of dorsomorphin analogues identifies selective VEGF and BMP inhibitors. *ACS Chem Biol*. 2010;5(2):245-53.
- [24] Gonzalez R, Lee JW, Snyder EY, Schultz PG. Dorsomorphin promotes human embryonic stem cell self-renewal. *Angew Chem Int Ed Engl*. 2011;50(15):3439-41.
- [25] Hao J, Sawyer DB, Hatzopoulos AK, Hong CC. Recent Progress on Chemical Biology of Pluripotent Stem Cell Self-renewal, Reprogramming and Cardiomyogenesis. *Rec Pat Regen Med*. 2011;1(3):263-74.
- [26] Sato N, Sanjuan IM, Heke M, Uchida M, Naef F, Brivanlou AH. Molecular signature of human embryonic stem cells and its comparison with the mouse. *Dev Biol*. 2003;260(2):404-13.
- [27] Beattie GM, Lopez AD, Bucay N, Hinton A, Firpo MT, King CC, et al. Activin A maintains pluripotency of human embryonic stem cells in the absence of feeder layers. *Stem Cells*. 2005;23(4):489-95.
- [28] James D, Levine AJ, Besser D, Hemmati-Brivanlou A. TGF beta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem. *Development*. 2005;132(6):1273-82.
- [29] Xiao L, Yuan X, Sharkis SJ. Activin A maintains self-renewal and regulates fibroblast growth factor, Wnt, and bone morphogenic protein pathways in human embryonic stem cells. *Stem Cells*. 2006;24(6):1476-86.
- [30] Vallier L, Alexander M, Pedersen RA. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. *J Cell Sci*. 2005;118(Pt 19):4495-509.
- [31] Xu RH, Sampsel-Barron TL, Gu F, Root S, Peck RM, Pan GJ, et al. NANOG is a direct target of TGF beta/Activin-mediated SMAD signaling in human ESCs. *Cell Stem Cell*. 2008;3(2):196-206.
- [32] Vallier L, Mendjan S, Brown S, Chng Z, Teo A, Smithers LE, et al. Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. *Development*. 2009;136(8):1339-49.
- [33] Ogawa K, Saito A, Matsui H, Suzuki H, Ohtsuka S, Shimosato D, et al. Activin-Nodal signaling is involved in propagation of mouse embryonic stem cells. *J Cell Sci*. 2007;120(Pt 1):55-65.
- [34] Levine AJ, Brivanlou AH. GDF3, a BMP inhibitor, regulates cell fate in stem cells and early embryos. *Development*. 2006;133(2):209-16.
- [35] Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, et al. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol*. 2000;227(2):271-8.

- [36] Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, et al. Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol.* 2001;19(10):971-4.
- [37] Brandenberger R, Wei H, Zhang S, Lei S, Murage J, Fisk GJ, et al. Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nat Biotechnol.* 2004;22(6):707-16.
- [38] Dvash T, Mayshar Y, Darr H, McElhaney M, Barker D, Yanuka O, et al. Temporal gene expression during differentiation of human embryonic stem cells and embryoid bodies. *Hum Reprod.* 2004;19(12):2875-83.
- [39] Ginis I, Luo YQ, Miura T, Thies S, Brandenberger R, Gerecht-Nir S, et al. Differences between human and mouse embryonic stem cells. *Dev Biol.* 2004;269(2):360-80.
- [40] Kim SJ, Cheon SH, Yoo SJ, Kwon J, Park JH, Kim CG, et al. Contribution of the PI3K/Akt/PKB signal pathway to maintenance of self-renewal in human embryonic stem cells (Retracted Article. See vol 580, pg 1529, 2006). *Febs Lett.* 2005;579(2):534-40.
- [41] Dvorak P. Basic fibroblast growth factor and its receptors in human embryonic stem cells. *Folia Histochem Cyto.* 2005;43(4):203-8.
- [42] Dvorak P, Dvorakova D, Koskova S, Vodinska M, Najvirtova M, Krekac D, et al. Expression and potential role of fibroblast growth factor 2 and its receptors in human embryonic stem cells. *Stem Cells.* 2005;23(8):1200-11.
- [43] Dailey L, Ambrosetti D, Mansukhani A, Basilico C. Mechanisms underlying differential responses to FGF signaling. *Cytokine Growth Factor Rev.* 2005;16(2):233-47.
- [44] Kang HB, Kim JS, Kwon HJ, Nam KH, Youn HS, Sok DE, et al. Basic fibroblast growth factor activates ERK and induces c-fos in human embryonic stem cell line MizhES1. *Stem Cells Dev.* 2005;14(4):395-401.
- [45] Li J, Wang GW, Wang CY, Zhao Y, Zhang H, Tan ZJ, et al. MEK/ERK signaling contributes to the maintenance of human embryonic stem cell self-renewal. *Differentiation.* 2007;75(4):299-307.
- [46] Bendall SC, Stewart MH, Menendez P, George D, Vijayaragavan K, Werbowetski-Ogilvie T, et al. IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. *Nature.* 2007;448(7157):1015-21.
- [47] Greber B, Lehrach H, Adjaye J. Fibroblast growth factor 2 modulates transforming growth factor beta signaling in mouse embryonic fibroblasts and human ESCs (hESCs) to support hESC self-renewal. *Stem Cells.* 2007;25(2):455-64.
- [48] Wang XF, Lin G, Martins-Taylor K, Zeng H, Xu RH. Inhibition of Caspase-mediated Anoikis Is Critical for Basic Fibroblast Growth Factor-sustained Culture of Human Pluripotent Stem Cells. *Journal of Biological Chemistry.* 2009;284(49):34054-64.
- [49] Eiselleova L, Matulka K, Kriz V, Kunova M, Schmidtova Z, Neradil J, et al. A Complex Role for FGF-2 in Self-Renewal, Survival, and Adhesion of Human Embryonic Stem Cells. *Stem Cells.* 2009;27(8):1847-57.

- [50] Burdon T, Stracey C, Chambers I, Nichols J, Smith A. Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. *Dev Biol.* 1999;210(1):30-43.
- [51] Kunath T, Saba-El-Leil MK, Almousaillekh M, Wray J, Meloche S, Smith A. FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment. *Development.* 2007;134(16):2895-902.
- [52] Stavridis MP, Lunn JS, Collins BJ, Storey KG. A discrete period of FGF-induced Erk1/2 signalling is required for vertebrate neural specification. *Development.* 2007;134(16):2889-94.
- [53] Battle-Morera L, Smith A, Nichols J. Parameters Influencing Derivation of Embryonic Stem Cells From Murine Embryos. *Genesis.* 2008;46(12):758-67.
- [54] Qi X, Li TG, Hao J, Hu J, Wang J, Simmons H, et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. *Proc Natl Acad Sci U S A.* 2004;101(16):6027-32.
- [55] Burdon T, Stracey C, Chambers I, Nichols J, Smith A. Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. *Dev Biol.* 1999;210(1):30-43.
- [56] Paling NR, Wheadon H, Bone HK, Welham MJ. Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. *J Biol Chem.* 2004;279(46):48063-70.
- [57] Chen S, Do JT, Zhang Q, Yao S, Yan F, Peters EC, et al. Self-renewal of embryonic stem cells by a small molecule. *Proc Natl Acad Sci U S A.* 2006;103(46):17266-71.
- [58] Chen S, Ding S, Yan F, Schultz P, inventors; Compounds that maintain pluripotency of embryonic stem cells patent US20100234400. 2010.
- [59] Ying QL, Wray J, Nichols J, Battle-Morera L, Doble B, Woodgett J, et al. The ground state of embryonic stem cell self-renewal. *Nature.* 2008;453(7194):519-23.
- [60] Smith A, Ying Q, inventors; Culture medium containing kinase inhibitors, and uses thereof patent US20080014638. 2008.
- [61] Aubert J, Dunstan H, Chambers I, Smith A. Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. *Nat Biotechnol.* 2002;20(12):1240-5.
- [62] Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med.* 2004;10(1):55-63.
- [63] Ogawa K, Nishinakamura R, Iwamatsu Y, Shimosato D, Niwa H. Synergistic action of Wnt and LIF in maintaining pluripotency of mouse ES cells. *Biochem Biophys Res Commun.* 2006;343(1):159-66.

- [64] Kielman MF, Rindapaa M, Gaspar C, van Poppel N, Breukel C, van Leeuwen S, et al. Apc modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. *Nat Genet.* 2002;32(4):594-605.
- [65] Pereira L, Yi F, Merrill BJ. Repression of Nanog gene transcription by Tcf3 limits embryonic stem cell self-renewal. *Mol Cell Biol.* 2006;26(20):7479-91.
- [66] Takao Y, Yokota T, Koide H. Beta-catenin up-regulates Nanog expression through interaction with Oct-3/4 in embryonic stem cells. *Biochem Biophys Res Commun.* 2007;353(3):699-705.
- [67] Wray J, Kalkan T, Smith AG. The ground state of pluripotency. *Biochem Soc Trans.* 2010;38(4):1027-32.
- [68] Bao SQ, Tang FC, Li XH, Hayashi K, Gillich A, Lao KQ, et al. Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature.* 2009;461(7268):1292-5.
- [69] Bao S, Tang F, Li X, Hayashi K, Gillich A, Lao K, et al. Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature.* 2009;461(7268):1292-5.
- [70] Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, et al. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. *Proc Natl Acad Sci U S A.* 2010;107(20):9222-7.
- [71] Nichols J, Smith A. Naive and primed pluripotent states. *Cell Stem Cell.* 2009;4(6):487-92.
- [72] Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, Smith A. Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol.* 2008;6(10):e253.
- [73] Silva J, Nichols J, Theunissen TW, Guo G, van Oosten AL, Barrandon O, et al. Nanog is the gateway to the pluripotent ground state. *Cell.* 2009;138(4):722-37.
- [74] Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, et al. New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature.* 2007;448(7150):196-9.
- [75] Pera MF, Tam PP. Extrinsic regulation of pluripotent stem cells. *Nature.* 2010;465(7299):713-20.
- [76] Tchieu J, Kuoy E, Chin MH, Trinh H, Patterson M, Sherman SP, et al. Female human iPSCs retain an inactive X chromosome. *Cell Stem Cell.* 2010;7(3):329-42.
- [77] Hanna JH, Saha K, Jaenisch R. Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues. *Cell.* 2010;143(4):508-25.
- [78] Lengner CJ, Gimelbrant AA, Erwin JA, Cheng AW, Guenther MG, Welstead GG, et al. Derivation of pre-X inactivation human embryonic stem cells under physiological oxygen concentrations. *Cell.* 2010;141(5):872-83.

- [79] Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*. 2008;132(4):567-82.
- [80] Hollnagel A, Oehlmann V, Heymer J, Ruther U, Nordheim A. Id genes are direct targets of bone morphogenetic protein induction in embryonic stem cells. *Journal of Biological Chemistry*. 1999;274(28):19838-45.
- [81] Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. *Science*. 2002;298(5593):601-4.
- [82] Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science*. 2002;298(5593):597-600.
- [83] Sato N, Sanjuan IM, Heke M, Uchida M, Naef F, Brivanlou AH. Molecular signature of human embryonic stem cells and its comparison with the mouse. *Dev Biol*. 2003;260(2):404-13.
- [84] Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*. 1998;95(3):379-91.
- [85] Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell*. 2003;113(5):643-55.
- [86] Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell*. 2003;113(5):631-42.
- [87] Liu Y, Labosky PA. Regulation of Embryonic Stem Cell Self-Renewal and Pluripotency by Foxd3. *Stem Cells*. 2008;26(10):2475-84.
- [88] Hanna LA, Foreman RK, Tarasenko IA, Kessler DS, Labosky PA. Requirement for Foxd3 in maintaining pluripotent cells of the early mouse embryo. *Gene Dev*. 2002;16(20):2650-61.
- [89] Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW, et al. A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. *Nature*. 1990;345(6277):686-92.
- [90] Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P. New type of POU domain in germ line-specific protein Oct-4. *Nature*. 1990;344(6265):435-9.
- [91] Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M, Hamada H. A Novel Octamer Binding Transcription Factor Is Differentially Expressed in Mouse Embryonic-Cells. *Cell*. 1990;60(3):461-72.
- [92] Yeom YI, Ha HS, Balling R, Scholer HR, Artzt K. Structure, expression and chromosomal location of the Oct-4 gene. *Mech Dev*. 1991;35(3):171-9.

- [93] Nishimoto M, Fukushima A, Okuda A, Muramatsu M. The gene for the embryonic stem cell coactivator UTF1 carries a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2. *Mol Cell Biol.* 1999;19(8):5453-65.
- [94] Tomioka M, Nishimoto M, Miyagi S, Katayanagi T, Fukui N, Niwa H, et al. Identification of Sox-2 regulatory region which is under the control of Oct-3/4-Sox-2 complex. *Nucleic Acids Res.* 2002;30(14):3202-13.
- [95] Zeng XM, Miura T, Luo YQ, Bhattacharya B, Condie B, Chen J, et al. Properties of pluripotent human embryonic stem cells BG01 and BG02. *Stem Cells.* 2004;22(3):292-312.
- [96] Niwa H, Toyooka T, Shimosato D, Strumpf D, Takahashi K, Yagi R, et al. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell.* 2005;123(5):917-29.
- [97] Ovitt CE, Scholer HR. The molecular biology of Oct-4 in the early mouse embryo. *Mol Hum Reprod.* 1998;4(11):1021-31.
- [98] Pan GJ, Chang ZY, Scholer HR, Pei DQ. Stem cell pluripotency and transcription factor Oct4. *Cell Res.* 2002;12(5-6):321-9.
- [99] Yeom YI, Fuhrmann G, Ovitt CE, Brehm A, Ohbo K, Gross M, et al. Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. *Development.* 1996;122(3):881-94.
- [100] Niwa H. Molecular mechanism to maintain stem cell renewal of ES cells. *Cell Struct Funct.* 2001;26(3):137-48.
- [101] Li M, Pevny L, Lovell-Badge R, Smith A. Generation of purified neural precursors from embryonic stem cells by lineage selection. *Curr Biol.* 1998;8(17):971-4.
- [102] Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. *Gene Dev.* 2003;17(1):126-40.
- [103] Zappone MV, Galli R, Catena R, Meani N, De Biasi S, Mattei E, et al. Sox2 regulatory sequences direct expression of a (beta)-geo transgene to telencephalic neural stem cells and precursors of the mouse embryo, revealing regionalization of gene expression in CNS stem cells. *Development.* 2000;127(11):2367-82.
- [104] Okumura-Nakanishi S, Saito M, Niwa H, Ishikawa F. Oct-3/4 and Sox2 regulate Oct-3/4 gene in embryonic stem cells. *Journal of Biological Chemistry.* 2005;280(7):5307-17.
- [105] Tokuzawa Y, Kaiho E, Maruyama M, Takahashi K, Mitsui K, Maeda M, et al. Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. *Mol Cell Biol.* 2003;23(8):2699-708.
- [106] Rodda DJ, Chew JL, Lim LH, Loh YH, Wang B, Ng HH, et al. Transcriptional regulation of Nanog by Oct4 and Sox2. *Journal of Biological Chemistry.* 2005;280(26):24731-7.

- [107] Kuroda T, Tada M, Kubota H, Kimura H, Hatano S, Suemori H, et al. Octamer and Sox elements are required for transcriptional cis regulation of Nanog gene expression. *Mol Cell Biol.* 2005;25(6):2475-85.
- [108] Nakatake Y, Fukui N, Iwamatsu Y, Masui S, Takahashi K, Yagi R, et al. Klf4 cooperates with Oct3/4 and Sox2 to activate the Lefty1 core promoter in embryonic stem cells. *Mol Cell Biol.* 2006;26(20):7772-82.
- [109] Ivanova N, Dobrin R, Lu R, Kotenko I, Levorse J, DeCoste C, et al. Dissecting self-renewal in stem cells with RNA interference. *Nature.* 2006;442(7102):533-8.
- [110] Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, et al. Nanog safeguards pluripotency and mediates germline development. *Nature.* 2007;450(7173):1230-4.
- [111] Suzuki A, Raya A, Kawakami Y, Morita M, Matsui T, Nakashima K, et al. Nanog binds to Smad1 and blocks bone morphogenetic protein-induced differentiation of embryonic stem cells. *Proc Natl Acad Sci U S A.* 2006;103(27):10294-9.
- [112] Vallier L, Mendjan S, Brown S, Chng Z, Teo A, Smithers LE, et al. Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. *Development.* 2009;136(8):1339-49.
- [113] Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell.* 2005;122(6):947-56.
- [114] Loh YH, Wu Q, Chew JL, Vega VB, Zhang W, Chen X, et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet.* 2006;38(4):431-40.
- [115] Mathur D, Danford TW, Boyer LA, Young RA, Gifford DK, Jaenisch R. Analysis of the mouse embryonic stem cell regulatory networks obtained by ChIP-chip and ChIP-PET. *Genome Biol.* 2008;9(8).
- [116] Sharov AA, Masui S, Sharova LV, Piao Y, Aiba K, Matoba R, et al. Identification of Pou5f1, Sox2, and Nanog downstream target genes with statistical confidence by applying a novel algorithm to time course microarray and genome-wide chromatin immunoprecipitation data. *BMC Genomics.* 2008;9:269.
- [117] Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, et al. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat Cell Biol.* 2007;9(6):625-35.
- [118] Wang J, Rao S, Chu J, Shen X, Levasseur DN, Theunissen TW, et al. A protein interaction network for pluripotency of embryonic stem cells. *Nature.* 2006;444(7117):364-8.
- [119] Goke J, Jung M, Behrens S, Chavez L, O'Keeffe S, Timmermann B, et al. Combinatorial Binding in Human and Mouse Embryonic Stem Cells Identifies Conserved Enhancers Active in Early Embryonic Development. *Plos Comput Biol.* 2011;7(12).

