

Intracellular Signaling Pathways Regulating Pluripotency of Embryonic Stem Cells

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Abstract: The cytokine LIF and its downstream effector STAT3 are essential for maintenance of pluripotency in mouse ES cells. The requirement for the transcription factor Oct3/4 for ES cell pluripotency is also well-documented. However, LIF is not involved in self-renewal of human ES cells, suggesting that other pathways must play an important role in this process. The importance of other signal transduction pathways, including BMP and Wnt signalings, as well as novel transcription factors such as Nanog, is now being recognized. We will review the rapid progress that has been made in identifying and dissecting the intracellular signaling pathways that contribute to self-renewal of pluripotent mouse and human ES cells.

Keywords: Growth factor, Cytokine, Regenerative medicine, Transcription factor, Crosstalk.

INTRODUCTION

Embryonic stem (ES) cells are derived from the inner cell mass, a population of cells in the blastocyst stage embryo that gives rise to all cells of the embryo proper. Once established *in vitro*, ES cells can be cultured indefinitely without losing their pluripotency, that is the ability to develop into any cell type in the body. These remarkable characteristics have made ES cells an extremely useful tool. The establishment of mouse ES cell lines in 1981 led to the development of the gene targeting technology used to generate knockout mice [1, 2], a technique that has quickly become a standard approach for investigating and modeling gene function. Moreover, since ES cells have unrestricted developmental capacity, they represent a promising source for cell transplantation therapies to treat various human diseases [3]. Since their isolation in 1998 [4], human ES cells have been coaxed to differentiate into such varied cell types as pancreatic β -cells, neurons, and cardiomyocytes, simply by changing the culture conditions in which the cells are grown. Future transplantation of these cells into patients suffering from diabetes, neurodegenerative diseases, and myocardial infarction holds a therapeutic promise.

However, the use of human ES cells as therapeutic treatment presents significant ethical and scientific problems. Since ES cells are derived from blastocyst stage embryos, the clinical use of human ES cells stirs up ethical objections against the destruction of human embryos. In addition, human ES cells must currently be grown and maintained on a feeder layer of mouse embryonic fibroblasts (MEFs) in a medium containing fetal bovine serum, which may lead to unexpected viral infection and/or cross-species contamination. Thus, for therapeutic applications, ES cells must be grown in a synthetic medium without factors or cells from animal-derived. Recently, Hwang *et al.* reported the establishment of patient-specific human ES cells

by means of somatic cell nuclear transfer from patient skin cells into donated oocytes [5]. They established those pluripotent cells on feeders from same patients. However their protocol still had to use calf serum to establish fibroblasts from patients.

In order to establish a defined serum free medium for human ES cells, and to generate ES-like cells from patients' somatic cells, it is essential to understand how ES cells maintain their pluripotency and ability to proliferate rapidly. In this review, we discuss recent progress in unraveling the intracellular signaling pathways that contribute to self-renewal of pluripotent mouse and human ES cells.

I. LIF/gp130/STAT3 (Fig. 1)

Mouse ES cells have historically been derived and maintained on a feeder layer of MEFs. However, conditioned media from MEFs can support the self-renewal of mouse ES cells, eliminating the need for a feeder layer. It was subsequently demonstrated that MEFs inhibit ES cell differentiation *via* production of the IL-6 family cytokine, leukemia inhibitory factor (LIF) [6, 7]. With the addition of recombinant LIF protein into the culture medium, mouse ES cells can be cultured without MEF feeder cells.

The receptor for LIF is a heteromeric complex consisting of gp130 and the LIF receptor (LIFR, also referred as to LIFR) [8]. The tyrosine kinase Janus kinase (JAK) binds constitutively to the intercellular domain of this receptor complex in its inactive form. Upon LIF binding, JAK kinase phosphorylates tyrosine residues of both gp130 and LIFR. Phosphorylation of Y765/812/904/914 of the intracellular domain of gp130 and Y976/996/1023 of LIFR recruits signal transducers and activators of transcription (STAT) 1 and STAT3 through their SH2 domains [9]. STAT proteins are then activated by JAK-mediated tyrosine phosphorylation to form homodimers and/or heterodimers and translocate into the nucleus, where they function as transcription factors [10]. LIF stimulation also induces other signaling pathways (described below).

Several groups have shown that STAT3 is important for maintenance of pluripotency of ES cells [11-14]. In

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particular, Mastuda *et al.* generated an inducible form of STAT3 by fusing it with the ligand-binding domain of the estrogen receptor, allowing for activation of STAT3 by tamoxifen administration. They reported that STAT3 activation is sufficient for self-renewal in the presence of fetal bovine serum [13]. One of important target genes of STAT3 is c-Myc, a helix-loop-helix/leucine zipper transcription factor [15]. However, LIF cannot support clonal expansion of mouse ES cells in the absence of serum [16]. This finding indicates that other factors required for ES cell proliferation and maintenance are present in serum or produced by MEFs.

Unlike mouse ES cells, LIF cannot promote self-renewal of human or monkey ES cells [17, 18]. Human ES cells express relatively low level of LIF signaling components (LIFR, JAK, and STAT3), and high level of suppressor of cytokine signaling (SOCS), which negatively regulate LIF signaling [19]. In monkey ES cells, depression of LIF signaling by dominant negative form of STAT3 did not affect their undifferentiated state [17]. Human and monkey ES cells seem to maintain the pluripotency in LIF/STAT3 independent manner.

II. BMP/Smad (Fig. 2)

Bone morphogenetic proteins (BMP) are members of the transforming growth factor (TGF- β) superfamily [20].

These secreted ligands bind to heterodimeric complexes of type I (ALK2, ALK3, ALK6) and type II (BMPRII, ActRII, ActRIIB) receptor tyrosine kinases. Binding of BMP triggers complex formation of the receptor components and facilitates phosphorylation of Smads, intracellular signal transduction molecules that fall into three categories: receptor-regulated Smads (R-Smads), cooperating Smad (Co-Smad) and inhibitory Smads (I-Smads). Upon BMP binding, R-Smads (Smad1, Smad5, and Smad8) are phosphorylated at two C-terminal serine residues and form heteromeric complexes with Smad4, the sole Co-Smad known in mammals. The Smad complexes then translocate to the nucleus and function as transcription factors. I-Smads (Smad6 and Smad7) suppress the Smad signaling pathway by inhibiting association between the receptors and R-Smads, competing with Smad1 for binding to the Co-Smad, and/or promoting ubiquitin-dependent degradation of receptors and R-Smads [21].

Ying *et al.* reported that BMP4 and LIF cooperate in the maintenance of pluripotency of mouse ES cells [16]. Under the serum-free culture conditions they used, LIF alone stimulated neural differentiation of ES cells. However, addition of BMP4 was able to suppress neural differentiation and maintain the undifferentiated state of mouse ES cells, even in the absence of serum. They also showed that BMP4 induced expression of inhibitor of differentiation (Id), an inhibitor for basic helix-loop-helix transcription factors

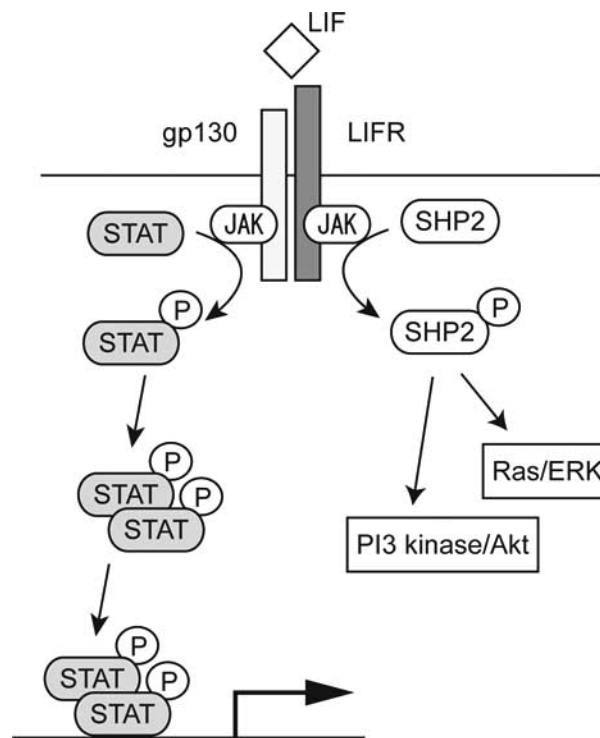


Fig. (1). Intracellular signaling pathways activated by LIF. Association of LIF with its heterodimeric receptor, which consists of LIFR and gp130, results in activation of several intracellular signaling pathways, including the STAT3 pathway, the Ras/ERK pathway, and the PI3 kinase pathway (see crosstalk section). The STAT3 pathway is crucial for the maintenance of pluripotency in mouse ES cells, but not in primate ES cells. LIF also activates the Ras/ERK and the PI3 kinase pathways (see Fig. 4).

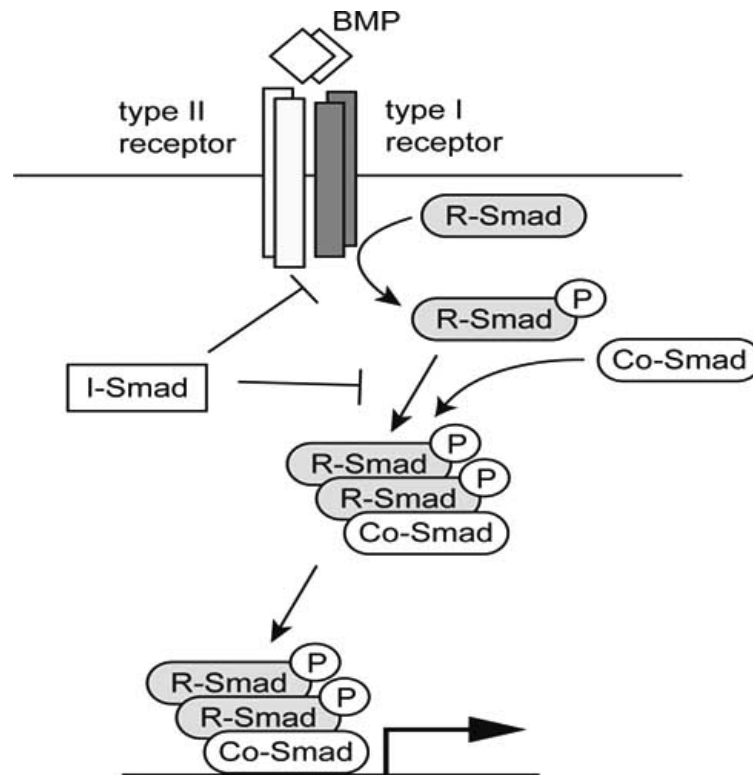


Fig. (2). Intracellular signaling pathway activated by BMP. Receptors of the TGF- superfamily of ligands consist of a heteromeric complex of type I and type II receptor serine/threonine kinases. Binding of BMP to the receptor induces phosphorylation of R-Smads by type I receptors. Phosphorylated R-Smads form complexes with Co-Smad and accumulate in the nucleus, where together they regulate gene transcription. I-Smads suppress the pathway *via* several mechanisms. In mouse ES cells, BMP4 can induce expression of Id and suppress neural differentiation. In human ES cells, in contrast, several groups reported that BMP4 induces differentiation.

known to be involved in many cell fate determinations, including neural differentiation [22]. When Id protein was overexpressed in mouse ES cells, LIF was able to maintain self-renewal of ES cells in the absence of either BMP4 or serum. Thus BMP4 appears to prevent neural differentiation of mouse ES cells through the induction of Id expression. By contrast, BMP4 alone facilitated mesodermal differentiation of mouse ES cells. These findings suggest that the self-renewal of mouse ES cells is achieved by a delicate balance between the two cytokines, LIF and BMP.

In human ES cells, BMP4 induces differentiation into mesoderm and ectoderm [23], whereas BMP2 promotes extraembryonic endoderm differentiation[23]. Repression of BMP signaling in human ES cells by adding the BMP antagonist Noggin and high doses of bFGF supports long-term self-renewal in the absence of serum and feeder cells [24].

III. Wnt/ β -Catenin/TCF (Fig. 3)

β -catenin is a cytoplasmic protein that functions in cell-cell adhesion by linking cadherins to the actin cytoskeleton.

It also acts as an intracellular signaling molecule of the canonical Wnt signaling pathway [25]. In the absence of Wnt activation, β -catenin is phosphorylated by a complex consisting of adenomatous polyposis coli gene (APC), Axin, and glycogen synthase kinase (GSK) 3. Phosphorylated β -catenin is degraded by the ubiquitin-proteasome system, thereby keeping the level of cytoplasmic β -catenin low. Upon binding of Wnt to its receptors, Frizzled and LRP5/6, GSK3 is inactivated through a poorly-understood mechanism involving the direct interaction of Axin with LRP5/6, and/or the action of an Axin-binding molecule, Dishevelled. As a result, β -catenin accumulates in the cytoplasm and travels to the nucleus, where it associates with lymphoid enhancer factor (LEF)/T-cell factor (TCF) transcription factors.

Aubert *et al.* showed that neural differentiation of mouse ES cells was attenuated by the activation of Wnt signaling by overexpression of Wnt1 or treatment with lithium chloride, an inhibitor of GSK3 [26]. Moreover, Wnt3a mutant mice display ectopic neural tube formation in the primitive streak of gastrulating embryo [27]. In addition, ES cells with a mutant form of APC show impaired ability to differentiate into the three germ layers [28]. Together, these

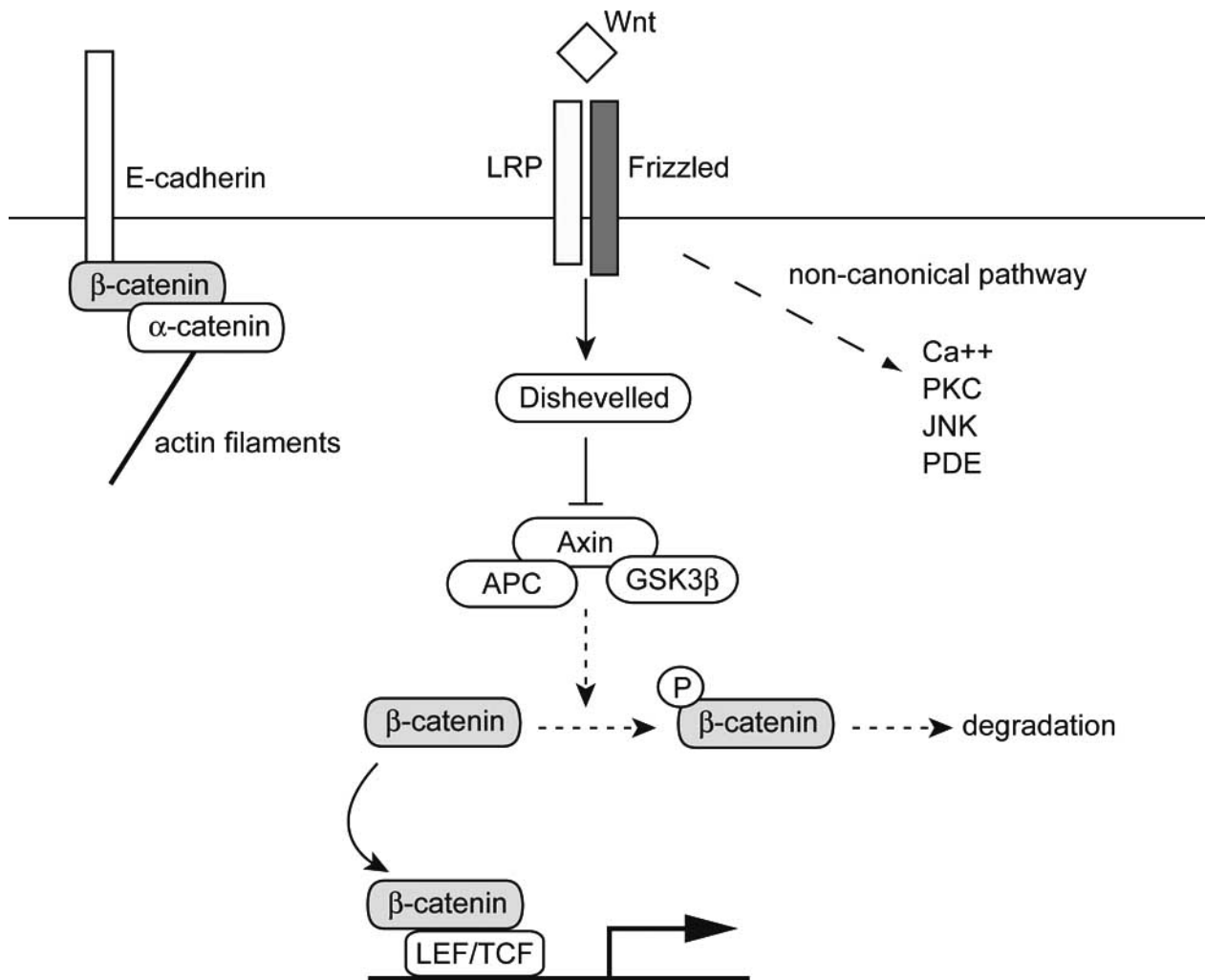


Fig. (3). Intracellular signaling pathway activated by Wnt. Wnt binds to its receptor, Frizzled, and coreceptor, LRP5 or LRP6. The downstream effector Dishevelled is then activated through mechanisms that are poorly understood. Activated Dishevelled inactivates the APC/Axin/GSK3 complex. Since this complex induces degradation of β -catenin in the absence of Wnt ligand, its inactivation results in the stabilization and accumulation of β -catenin protein in the nucleus. β -catenin binds to and activates LEF/TCF transcription factors. In both mouse and human ES cells, the Wnt/ β -catenin pathway may promote self-renewal.

data suggest that Wnt signaling may suppress differentiation in early embryos and in ES cells.

Sato *et al.* compared the gene expression profile of undifferentiated and differentiated human ES cells in detail, and revealed the enrichment of Frizzled 5 in undifferentiated ES cells [29]. They also reported that 6-bromoindirubin-3'-oxime (BIO), a newly identified pharmacological inhibitor of GSK3, could maintain mouse ES cell pluripotency in the absence of LIF [30]. Noteworthy, BIO also sustained human ES cells at the undifferentiated state, and maintained the expression of Oct3/4, Rex1, and Nanog. Considering LIF has little effect on human ES cells, BIO would be useful to prove the culture techniques of human ES cells.

Wnt also accelerates the proliferation of stem cells in the intestinal, epidermal and hematopoietic systems; it may represent a common factor controlling stem cell proliferation [31]. However, it has been reported that Wnt facilitates neural differentiation of ES cells and induces the expression of mesoderm marker, Brachyury [32]. Additional

experiments are required to clarify the precise function of Wnt/ β -catenin signaling in ES cells and other stem cell systems.

Wnt can signal independently of β -catenin through so-called non-canonical pathways [33]. These include calcium flux, JNK and both small and heterotrimeric G proteins. In F9 mouse embryonic carcinoma cells, Wnt5A can activate cGMP-specific phosphodiesterase through heterotrimeric G proteins [34]. The roles of these non-canonical Wnt pathways in pluripotency remain elusive.

IV. Phosphatidylinositol 3 (PI3) Kinase (Fig. 4)

PI3 kinases are lipid kinases that catalyze the phosphorylation of inositol phospholipids at the third carbon position of the inositol ring [35]. They are divided into three major classes according to substrate specificity, amino acid sequence, and homology of lipid kinase domains. Class 1A PI3 kinases are heterodimers consisting of an

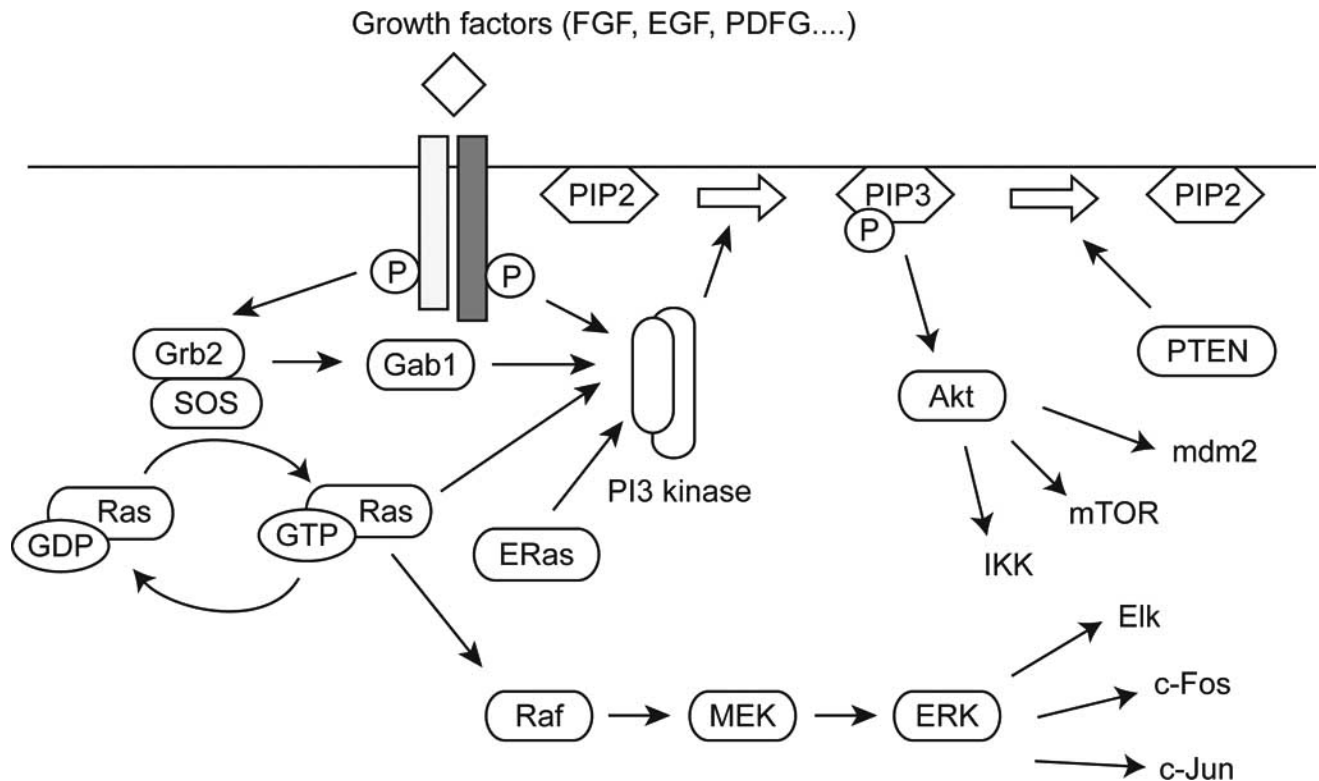


Fig. (4). Activation of the Ras/ERK pathway and PI3 kinase pathway by growth factors. Binding of growth factors to their receptors induces autophosphorylation of receptors and/or phosphorylation of receptor-associated proteins. The adaptor protein Grb2 binds to the phosphorylated tyrosines through its SH2 domains and activates the Ras/ERK pathway through the GTP-GDP exchange factor SOS. Activation of the Ras/ERK pathway induces differentiation in mouse ES cells. The PI3 kinase pathway can be activated *via* three routes. First, Gab1 can bind to Grb2, resulting in tyrosine phosphorylation and activation of the PI3 kinase pathway. Second, the PI3 kinase-regulatory subunit p85 can bind to a phosphorylated tyrosine residue of the receptor. Alternatively, activated Ras can induce membrane localization and activation of the p110 catalytic subunit of PI3 kinase. In addition, the PI3 kinase pathway is constitutively activated by ERas in mouse ES cells. PTEN is a negative regulator of the PI3 kinase pathway. The PI3 kinase pathway can promote self-renewal of mouse and human ES cells, possibly by suppression of the ERK pathway.

adaptor/regulatory and a catalytic subunit. The regulatory subunits come in seven isoforms generated by alternative splicing from three genes (p85 , p85 , and p55), while there are three isoforms of the catalytic subunit (p110 , p110 , and p110). Activation of the class 1A PI3 kinases is induced by many different receptor tyrosine kinases for growth factors, such as FGF, EGF, and PDGF, and leads to generation of the second messenger phosphatidylinositol-3,4,5-tris-phosphate (PIP₃). A serine/threonine kinase, Akt1, binds to PIP₃ through its pleckstrin homology (PH) domain and is translocated to the inner cell membrane, where it is phosphorylated and activated by another serine/threonine kinase PDK1. Activated Akt1 modulates the function of numerous substrates, such as Mdm2, IKK, and mammalian target of rapamycin (mTOR), and elicits various cellular responses, including proliferation and suppression of cell death. PI3 kinase is also known to act as a downstream effector of Ras [36].

Treatment of mouse ES cells with LY294002, a potent PI3 kinase inhibitor, suppressed progression of cells from the G1 to S phase and decreased cell proliferation [37]. Targeted disruption of phosphatase and tensin homologue deleted on chromosome ten/PTEN/a negative regulator of the PI3 kinase pathway through PIP₃ dephosphorylation,

promotes ES cell proliferation and tumorigenicity [38]. Thus, the PI3 kinase pathway is likely to be a crucial regulator of ES cell proliferation. In addition, the PI3 kinase pathway may be involved in the maintenance of pluripotency in both mouse and human ES cells [39, 40].

V. Ras/Raf/ERK (Fig. 5)

Ras proteins belong to a superfamily of low molecular weight GTP-binding proteins, and control cell proliferation and differentiation in variety of cells [41, 42]. Like other GTP-binding proteins, Ras exists in two states – an active GTP-bound state and an inactive GDP-bound state. The conformational changes from the inactive to the active state are mediated by Ras-GTP exchange factors.

Many different growth factors are able to activate Ras signaling by binding to receptor tyrosine kinases, resulting in autophosphorylation of tyrosine residues on the receptors. SH2 domain-containing tyrosine phosphatase (SHP) 2 and growth factor receptor binding protein (Grb) 2 then bind to the phosphotyrosines on the receptors and activate Ras through Ras-GTP exchange factors, son of sevenless (SOS), which is constitutively associated with Grb2. Activated Ras binds to many downstream effector proteins, including Raf

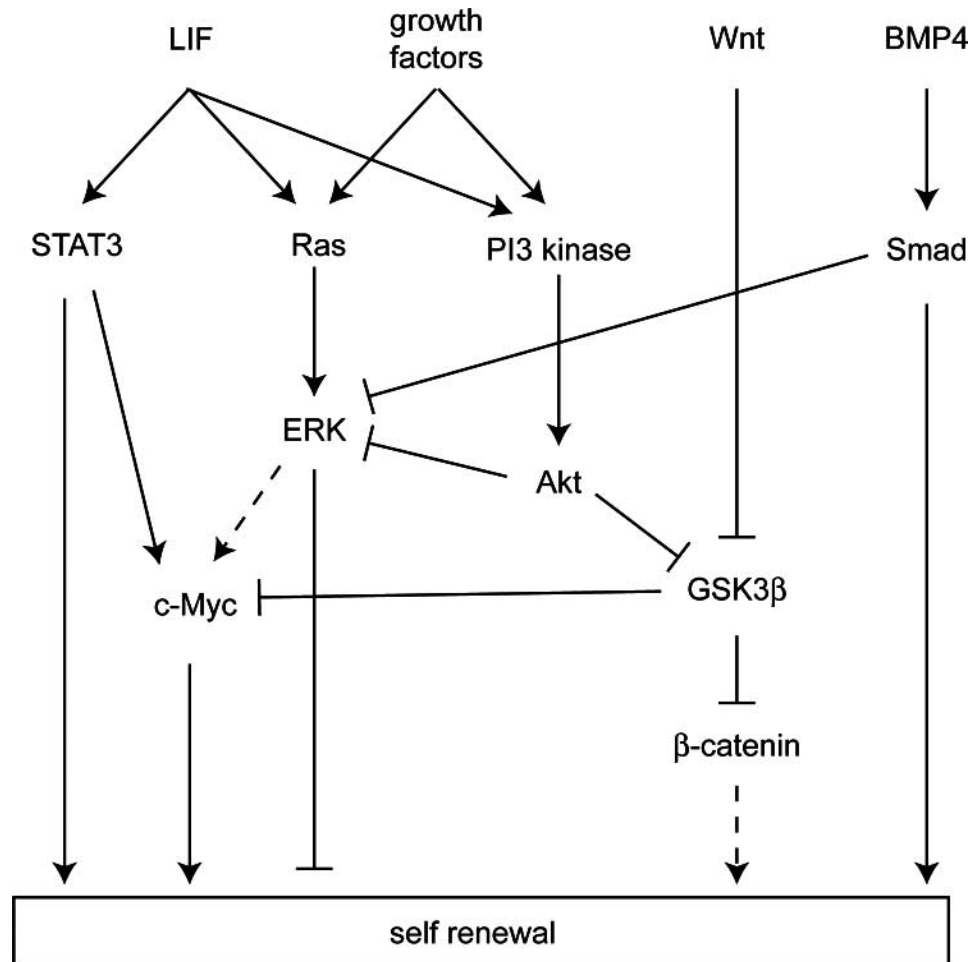


Fig. (5). Potential crosstalk between intracellular signaling pathways in mouse ES cells. STAT3 induces c-Myc expression and then c-Myc activated several target gene for self-renewal. GSK3 inactivates c-Myc by phosphorylation and proteasome-mediated degradation. However, it is not clear whether c-Myc is the only downstream effector of LIF/STAT pathway accounted for pluripotency. BMP/Smad pathway prevents differentiation by inducing target genes like Id, and their signal also suppress the ERK pathway through indirect mechanisms. The PI3 kinase pathway inhibits the ERK signaling probably thorough the phosphorylation of Raf by Akt. Wnt stimulation and inhibition of GSK3 was reported to enhance self-renewal of mouse and human ES cells. Maintenance of pluripotency by Wnt signaling may be achieved by accumulation of c-Myc and/or β -catenin. c-Myc has been identified as one of the target genes of Ras/ERK pathway in fibroblasts [75, 76]. Whether this is also the case in ES cells is uncertain.

serine/threonine kinases, which activate extracellular signal-regulated kinase (ERK). This pathway leads to phosphorylation and activation of transcription factors, such as c-Jun, c-Fos, Ets, and Elk [43].

Although the Ras/ERK pathway promotes proliferation and survival of many different types of cells, multiple lines of evidence indicate that the pathway promotes differentiation and suppresses proliferation of mouse ES cells. For example, the tyrosine residue in gp130 required for activation of Ras/ERK pathway is dispensable for maintaining self-renewal of mouse ES cells [13]. In addition, the ERK inhibitor PD98059 promotes efficient derivation of ES cells from blastocysts [44]. Grb2-null ES cells fail to differentiate into endoderm lineages [45], while ectopic expression of the active form of HRas in ES cells results in massive differentiation into primitive endoderm lineages [46]. Taken together, these results suggest that the

Ras/ERK pathway promotes differentiation of and suppresses self-renewal of mouse ES cells.

VI. Crosstalk Between Intracellular Signaling Pathways

Some of the intracellular signaling pathways mentioned above are known to engage in crosstalk with one other. In addition to STAT, LIF stimulation induces other signaling pathways through effector protein, SHP2, which can bind to Y757 of the intracellular domain of gp130 (Y118 in the cytoplasmic domain) and Y969 of LIFR (Y115 in the cytoplasmic domain) [9, 47]. When phosphorylated by JAK, SHP2 binds the adaptor protein Grb2 and activate the Ras/ERK signaling pathway. Phosphorylated SHP2 also binds Grb2-associated binder (Gab), activating the PI3 kinase pathway. The activation of SHP2 is thought to be dispensable for ES cell self-renewal, but is required for proper differentiation [44, 48].

Myc is reported to be one of the STAT3 target genes participating in self-renewal and maintenance of pluripotency in mouse ES cells [15]. Forced expression of stable c-Myc rendered self-renewal without LIF, whereas dominant negative form of c-Myc induced differentiation even in the LIF existence. GSK3 negatively regulates c-Myc activity by phosphorylation following degradation by the proteasome system [49]. Thus c-Myc is a common target both LIF and Wnt signaling pathways.

The PI3 kinase pathway is activated by exogenous factors such as insulin, but also activated endogenously by ERas (ES cell-expressed Ras), a novel small GTP binding protein specifically expressed in mouse ES cells [50]. Although its identity with HRas is less than 40%, the five domains essential for Ras function, as well as the CAAX motif required for plasma membrane localization, are highly conserved. However, ERas lacks several amino acids that are conserved among other Ras family proteins and essential for GTPase activity. As a consequence, ERas shows high GTP affinity and is constitutively active. Among known downstream effectors of Ras, including Raf1, BRas, RalGDS, and PI3 kinase, ERas specifically binds to and activates PI3 kinase.

The PI3 kinase pathway [39] and BMP4 [51] appear to maintain the undifferentiated state of mouse ES cells *via* inhibition of ERK and both ERK and p38, respectively. Suppression of the ERK activity by the PI3 kinase pathway was also observed in ES cells deficient in the catalytic subunit p85 [52] or PDK1, which activates Akt [53]. PI3 kinase pathway inhibits ERK signaling probably through the phosphorylation of Raf by Akt [54]. The PI3 kinase pathway can activate the Wnt/ -catenin pathway *via* inhibition of GSK3 by Akt, but its role in ES cells is not clear [39].

Another crosstalk of each signal components also reported. For instance, Wnt was reported to inhibit neuronal differentiation in ES cells by inducing BMP expression [41]. LEF/TCF1, a transcription factor downstream of Wnt signaling, complexes with Smad4, Co-Smad in the BMP pathway, to cooperatively control gene expression in mouse ES cells [42].

Crosstalk between signaling pathways has also been described in neural progenitor and stem cells. For example, cooperation between LIF and BMP2 signaling is mediated by interaction between STAT3 and Smad1, which form a complex together with p300, resulting in astrocyte induction [55]. BMP4 induces smooth muscle differentiation *via* Smads in a low cell density culture, whereas it induces glial cell differentiation *via* mTOR and STAT3 at a higher cell density [56]. Whether such crosstalk between pathways also functions in ES cells or not is currently unknown.

In human ES cells, the best known factor promoting self-renewal is fibroblast growth factor (Fgf) 2 [24]. Exogenous Fgf2 is capable of maintaining human ES cells in the absence of serum and feeder cells. Binding of Fgf to its receptor and heparin leads to receptor autophosphorylation and activation of intracellular signaling cascades, including the Ras/ERK pathway, the PLC /Ca²⁺ pathway, and the PI3 kinase pathway [57]. Fgf2 may promote self-renewal of human ES cells by activating the PI3 kinase pathway [40].

VII. Transcription Factors Controlling Self-Renewal of ES Cells

Oct3/4 and Nanog are the two well-known homeobox transcription factors that are specifically expressed in mouse ES cells and early embryos, and are essential for maintaining pluripotency [58]. Knockdown experiments using siRNA indicated that the two homeoproteins are also indispensable in human ES cells [59]. The intracellular signaling pathways described above are likely to regulate these transcription factors to determine the fate of ES cells. However, the precise mechanisms by which these factors are regulated remain elusive.

Oct3/4 (also known as POU5F1) is specifically expressed in ES cells, early embryos, and germ cells [60, 61]. Oct3/4-deficient embryos die at peri-implantation stages of development [62]. Although Oct3/4-null embryos reach blastocyst stage, the inner cell mass of these mutants only produces differentiated cells of trophoblast lineages when cultured *in vitro*. Inactivation of Oct3/4 in mouse ES cells also results in trophoblast differentiation [63]. In contrast, overexpression of Oct3/4 in ES cells induces differentiation into primitive endoderm and mesoderm lineages [63]. Hence, the expression level of Oct3/4 is an important determinant of cell fates in mouse ES cells.

Nanog is also specifically expressed in pluripotent cells [64, 65]. Nanog null embryos at E5.5 show disorganization of extraembryonic tissues with no discernible epiblast or extraembryonic ectoderm [64]. Nanog-deficient blastocysts appear to be normal, but the inner cell mass fails to generate epiblast and only produces parietal endoderm-like cells when cultured *in vitro*. Similarly, ES cells lacking Nanog preferentially differentiate into extraembryonic endoderm lineages even in the presence of LIF. Importantly, overexpression of Nanog allows mouse ES cells to self-renew without LIF. Thus, Nanog blocks primitive endoderm differentiation and actively maintains pluripotency.

VIII. Epigenetic Modification

Epigenetic modifications, including CpG methylation and histone modifications, regulate gene transcription and are also important in the maintenance of pluripotency. ES cells having mutation in DNA methyltransferase (Dnmt) can differentiate but show abnormal gene expression [66]. CpG binding protein (CGBP) has a unique DNA-binding specificity for unmethylated CpG dinucleotides. Mouse ES cells lacking the CGBP show reduced levels of genomic methylation and maintenance DNA methyltransferase activity. The cells remain undifferentiated even in LIF-withdrawal [67]. CpG dinucleotides of Oct3/4 and Nanog gene are hypomethylated in undifferentiated human ES cells, whereas methylation progresses during neural differentiation [68]. In mouse development, the upstream regulatory region of Oct3/4 gene is unmethylated in blastocysts and in epiblast at E6.25 and methylation initiates at E6.5 [69]. The methylation pattern of Oct3/4 and Nanog is highly comparable with their expression pattern, and the methylation would suppress their transcription in differentiated cells. *In vitro* fusion with ES cells or treatment with demethylating agent, 5-azacytidine, could erase, at least in part, epigenetic status of differentiated cells [70, 71].

CONCLUSION

Our understanding of the molecular mechanisms underlying pluripotency of ES cells has progressed remarkably in the last few years [72]. In addition to the factors we discussed in this review, it is highly likely that other factors and pathways are also involved, including miRNA [73] and Bcl2 [74]. However, some data are still controversial. As described above, Wnt was reported as a self-renewal factor by some groups, but as a differentiation initiation factor by others. This discrepancy may at least in part be due to the different ES cell lines used in the different experiments.

Many unsolved questions remain. For instance, what is the relationship between the intracellular signaling pathways and the transcription factors described in this review? Do mouse and human ES cells share common pathways to maintain pluripotency? To answer these questions and to further the possibility of medical applications of stem cell technology, further studies into the mechanisms underlying the maintenance of pluripotency are essential.

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