CONTROLLING THE STEM CELL COMPARTMENT AND REGENERATION IN VIVO: THE ROLE OF PLURIPOTENCY PATHWAYS

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I. INTRODUCTION

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have the capacity to self-renew, and the differentiation of these cells can yield over 200 diversely functional cell types. The ability of pluripotent cells to differentiate and produce a potential source of somatic cells makes these cells an obvious choice for regenerative medicine. Unfortunately, before the potential of pluripotent cells can be harnessed for clinical application, it is essential that we understand the mechanisms involved in maintaining pluripotency and inducing differentiation. The recent discovery of somatic cell reprogramming with key pluripotency factors has provided essential information on the role of transcription factors in pluripotency maintenance. These pluripotency factors are thought to be capable of initiating most, if not all, of the necessary developmental signaling pathways required for somatic cell reprogramming. The generation of these reprogrammed iPS cells has opened up the prospect of deriving patient-specific cells for regenerative medicine and may resolve the ethical issues and the immune rejection risks associated with the use of human ES cells.

While the generation of iPS cells and the identification of key pluripotency factors has been a breakthrough in understanding pluripotency, somewhat less is known of the signaling networks surrounding these key transcription factors. Without a clearer understanding of the signaling network involved in pluripotency maintenance and also differentiation, the use of iPS cells and ES cells in cellular therapy will be limited, as will our capacity to modulate existing stem cells in situ. It is therefore essential that further mechanistic and functional studies are carried out, which will inevitably continue to identify factors that are capable of defining stemness and mediating reprogramming in culture. This also raises the much wider question of the relevance of these mechanisms in adult stem cells and to normal physiology, either in maintaining tissue homeostasis, or more critically in mediating the repair response to disease and injury. Here, we will review the potential path-
ways that may be downstream of pluripotency factors identified in ES cells and iPS cells (152, 255, 261) and also examine their functional relevance in adult somatic stem cells. Such an improved understanding will not only enhance our fundamental understanding of the regulation of tissue maintenance, but may also open the possibility of directly manipulating these pathways to mediate efficient regeneration following disease or injury.

II. PLURIPOTENCY FACTORS

A. Embryonic Stem Cells

As described above, ES cells are pluripotent cells that are capable of unlimited self-renewal and are able to differentiate into all three embryonic lineages. To maintain their
ability to self-renew and remain undifferentiated, ES cells require the cooperation of several regulatory factors, including a unique set of transcription factors (26, 117), Polycomb complexes (27, 36), microRNAs (164), and histone modification enzymes (23). Although the precise role of these mechanisms is still unclear, several studies have identified the core transcription factors that are necessary for pluripotency in ES cells. Oct4 and Nanog are two homeodomain transcription factors and were the first to be identified as essential for early embryo development and pluripotency maintenance in ES cells (37, 168, 176). In addition, Sox2, an HMG-box transcription factor, which heterodimerizes with Oct4, has also been shown to be central to the transcriptional network regulating pluripotency in mouse ES cells (26). All three of these transcription factors act as either transcriptional activators or repressors and share a substantial fraction of their target genes (26, 155). The ability of these factors to activate or repress genes allows them to maintain pluripotency by promoting the expression of downstream self-renewal genes while simultaneously repressing the activity of differentiation-promoting genes. These factors also function interdependently and demonstrate an autoregulatory feedback loop which enables them to maintain the ES cell transcriptome through a tight regulatory network (FIG. 1).

Many other transcription factors have been identified to closely cooperate with these three essential pluripotency factors including Sal14, Dax1, Essrb, Tcf4, Klf4, Tbx3, Tcl1, Rif1, Nac1, and Zfp281 (40, 43, 102, 117, 155, 257). These transcription factors also regulate each other, forming a complicated transcriptional regulatory network in ES cells (291). Essrb, Rif1, Zic3, and Tcl1 are also primary targets of both Oct4 and Nanog (148, 155). In addition, RNAi-mediated knockdown has confirmed that eight genes, including Nanog, Oct4, and Sox2, are essential for maintaining an undifferentiated state in ES cells (102). Expression profile analysis has also identified developmental genes (Otx2, Pitx2, and Sox18) regulated by these eight factors, which induce ES cell differentiation when overexpressed (102).

### B. Induced Pluripotent Stem Cells

From the above, it is clear that ES cell studies have implicated a number of factors in pluripotency, the requirement...
for which has subsequently been confirmed by reprogramming somatic cells with specific genes to generate iPS cells (FIG. 2). For example, ectopic expression of Oct4, Sox2, c-Myc, and Klf4 together was shown to be sufficient to reprogram both mouse embryonic and adult fibroblasts to a pluripotent state (160, 186, 237, 263). These reprogrammed cells were able to yield live born chimeras and were also able to produce iPS cell progeny through germline transmission. In addition, they displayed similar epigenetic and transcriptional profiles compared with ES cells, such as their status with respect to DNA methylation, histone H3 methylation, X chromosome activation, and global gene expression profiles (160, 186, 263). These studies also revealed reactivation of the endogenous Oct4, Sox2, and Nanog genes, all of which are markers for undifferentiated ES cells. Oct4, Sox2, c-Myc, and Klf4 have also been shown to successfully reprogram human dermal fibroblasts (191, 236), whereas others have shown that Oct4, Sox2, Nanog, and Lin28 were sufficient to establish pluripotent cells from human somatic cells (283). In all three of these studies, the transcriptional and epigenetic profiles were similar to human ES cells and the iPS cells formed derivatives of all three germ layers when differentiated in vitro and in teratoma assays.

In addition to the groups of pluripotency factors used to reprogram iPS cells, the levels and ratios of the reprogramming factors have a critical role in the efficiency of reprogramming. For example, increasing Oct4 levels increases reprogramming efficiency, whilst increasing Sox2, Klf4, or c-Myc individually causes a significant decrease in reprogramming efficiency (47). The optimal ratio of the reprogramming factors will vary depending on the cell type used and also the endogenous expression of any one of the reprogramming factors within the starting cell population. A summary of some of the reprogramming scenarios that have been used successfully in both mouse and human are given in TABLE 1.

Although the mechanisms behind reprogramming somatic cells back to a pluripotent state are largely unknown, recent work has shown that the reprogramming process with defined factors is a gradual, time-dependent process, and studies by Brambrink et al. (28) and Stadfield et al. (227) have identified the sequence of key events necessary for the reprogramming of mouse embryonic fibroblasts. The minimal time for full reprogramming of mouse somatic cells has been defined as 8–12 days, and reprogramming appears to follow a sequential path consisting of early and late events. The reprogramming factors progressively reestablish the core circuitry of pluripotency, and as reprogramming progresses, the properties of pluripotent stem cells begin to appear.

III. THE EPIGENETIC REGULATION OF PLURIPOTENCY

Gene regulation during pluripotency also requires epigenetic changes to chromatin, and it has been well established that ES cells and differentiated cells have significantly different epigenetic signatures (22, 23). Pluripotent cells require a dynamic and transcriptionally permissive chromatin structure whereby structural proteins are loosely associated, enabling rapid reorganization of the chromatin structure in response to differentiation signals (166). Lineage-specific genes are therefore poised for transcriptional activation but are held in check by repressive chromatin

![Diagram](image-url)

**FIGURE 2.** Schematic of iPS cell derivation. Adult somatic cells can be reprogrammed to pluripotent iPS cells by introducing a specific combination of key transcription factors such as the Yamanaka factors, Oct4, Sox2, Klf4, and c-myc. iPS cells display similar characteristics to ES cells such as DNA methylation and expression profiles and are also able to differentiate into advanced derivatives of all three primary germ layers.
methylation until the appropriate signals are received (23). The epigenetic status of iPS cells and their cells of origin are also markedly different, suggesting that reprogramming involves dynamic chromatin changes, and it has been demonstrated that during reprogramming the epigenetic status progresses from a somatic to a pluripotent level (120).

This specialized chromatin state is established partly by the activity of ATP-dependent nucleosome remodeling complexes, such as Swi2/Snf2. A number of ATP-dependent nucleosome remodeling complexes have been shown to be important for ES cell self-renewal and differentiation, and recent studies have demonstrated that treatment with various chromatin modifiers can improve the frequency of iPS cell generation (93, 94). ES cells contain a family of functionally and structurally specialized chromatin remodeling complexes that are critical for the self-renewal of ES cells and maintain the stem cell state (90). Several BAF complexes, including a unique complex, esBAF, are expressed in ES cells, and ES cells depleted of various known subunits of BAF have been shown to have defects in gene regulation, self-renewal, and pluripotency (72, 277). The catalytic ATP-Pase SWI/SNF subunit Brg acts as the catalytic subunit of esBAF and has been identified as an essential factor in nuclear reprogramming (87). esBAF plays a direct role in mediating the gene regulatory function of several core pluripotency factors. The Brg subunit selectively regulates the expression of several factors of the core network as well as interacting directly with Oct4, Sox2, and Nanog (90). Brg also interacts with several other proteins that have been implicated in ES cell maintenance such as Rif1, Sall4, Dppa2, and Dppa4, and it is thought that the surface of esBAF may be tailored for interaction with factors specifically found in ES cells. esBAF also functions to repress pluripotency genes such as Oct4, Sox2, and Nanog; this inhibition is thought to refine their levels and maintain pluripotency. In addition to BAF, several other chromatin modifiers have recently been identified as vital factors in maintaining ES cell pluripotency, such as Chd1 (73), the histone deacetylase NuRD (106, 292), and Tip60-p400 (65). Similarly to BAF, these factors interact and regulate the core pluripotency transcription factors of ES cells.

As described above, pluripotency factors and epigenetic regulators engage in cross-talk with one another to maintain pluripotency. Several pluripotency factors that are regulated by epigenetic regulators also regulate the genes encoding the epigenetic control factors; for example, Oct4, Sox2, and Nanog. It has been demonstrated that during reprogramming the epigenetic status progresses from a somatic to a pluripotent level (120).

### Table 1. Examples of the various combinations of transcription factors and cell types that have been used in the production of iPS cells

<table>
<thead>
<tr>
<th>Species</th>
<th>Cell Type</th>
<th>Factors</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human HNF</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>HDF</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>237, 289</td>
<td></td>
</tr>
<tr>
<td>HFLS</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>HFr</td>
<td>Oct4, Sox2, Klf4</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>107, 269</td>
<td></td>
</tr>
<tr>
<td>CD34+ blood cells</td>
<td>Oct4, Sox2, Nanog, LIN28</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>Adult fibroblasts</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>Foreskin fibroblasts</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>H1F cells</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>MSCs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>Adult fibroblasts</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>HDFs</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>BJ cells</td>
<td>Oct4, Sox2, Klf4</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Mouse MEFs and tail tip fibroblasts</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>MEFs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>Tail tip fibroblasts and hepatocytes</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>Hepatocytes and gastric epithelial cells</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>MEFs and tail tip fibroblasts</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>MEFs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>NSCs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>NPCs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>MEFs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>MEFs and NSCs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>MEFs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
<tr>
<td>NSCs</td>
<td>Oct4, Sox2, c-Myc, hTERT, SV40 large T</td>
<td>146, 283</td>
<td></td>
</tr>
</tbody>
</table>
Sox2, and Nanog coregulate certain genes encoding chromatin remodeling and histone-modifying complexes, such as Ehmt1, SMARCAD1, MYST3, and SET in mouse and human ES cells (26). Also, pluripotency factors interact with histone-modifying enzymes and chromatin remodeling complexes; for example, Nanog and Oct4 interact directly and indirectly with the polycomb group (YY1, Rnf2, and Rybp) and SWI/SNF chromatin remodeling (BAF155) factors and KAP1 (Tif1β) (257). In addition, several other genes regulated by pluripotency factors are subjected to epigenetic regulation. For example, two downstream targets of Oct4, Jmjd1a and Jmjd2c, both of which are histone demethylases, positively regulate several pluripotency-associated genes including Tcl1, Tcfct2ll, Zfp57, and Nanog (155, 156).

IV. IPS CELLS IN THERAPY

The reprogramming of human somatic cells to a pluripotent state has significant implications for the generation of patient-specific, genetically compatible cells for transplantation. Table 2 lists a number of disease models in which iPS cells have already been used as a potential therapy. iPS cells have been generated from the cells of various mouse tissues including blood (85), liver (8), stomach (8), pancreas (227), brain (61, 119, 221), and the intestine and adrenals (262) as well as also human fibroblasts (158, 191, 236), keratinocytes (1), and blood progenitor cells (154). Recent studies using iPS cells to alleviate the symptoms of Parkinson’s disease and sickle cell anemia in mouse models have demonstrated the potential of these cells in cellular therapy (86, 264). Furthermore, Raya et al. (203) reprogrammed genetically corrected Fanconi anemia fibroblasts and obtained genetically corrected Fanconi anemia-specific iPS cells that can give rise to phenotypically normal hematopoietic progenitors of the myeloid and erythroid lineages. In addition to neural and blood diseases, iPS cells have been differentiated into many types of cardiovascular cells, including arterial, venous, and lymphatic endothelial cells as well as cardiomyocytes (172, 285). Another potential therapeutic use of iPS cells is in the treatment of infertile patients. Primordial germ cells (PGCs) can be generated through in vitro differentiation of human iPS and ES cells, and these in vitro differentiated PGCs show gene expression similarities to in vivo PGCs (192). If these PGCs can be further differentiated into sperm and oocytes, it will be valuable for infertile patients. While these studies demonstrate the potential therapeutic application of iPS cells, our current limited understanding of the reprogramming process hinders the ultimate goal of using these cells in cellular therapy.

V. PLURIPOTENCY PATHWAYS

Signal transduction pathways are critical in maintaining pluripotency and controlling the lineage-specific differentiation of stem cells. Although ES cell research began more than 20 years ago, the regulatory network of ES cell maintenance is still largely unknown. Several extrinsic growth factors support the pluripotency of ES cells in vitro, with mouse ES cells usually being cultured in the presence of leukemia inhibitory factor (LIF) and bone morphogenetic proteins (BMPs). LIF is thought to activate STAT3 signaling pathways to maintain pluripotency, whilst BMPs act to inhibit differentiation by activating members of the Id gene (inhibition of differentiation) family (177, 279). Importantly, recent work has also indicated that ES cells can be cultured in the absence of LIF and BMP. These ES cells represent a ground state, a basal proliferative state that is free of epigenetic restriction and has minimal requirements for extrinsic stimuli (280). In this state, the pluripotency network, headed by Oct4/Sox2, Nanog, and KLF2/KLF4, is intrinsically stable but extremely sensitive to destabilization by exogenous signals, with ES cells requiring the presence of MEK and GSK3 inhibitors to suppress innate differentiation stimuli and maintain the stability of the ground state (84, 271).

The commitment of ES cells to a specific lineage is therefore regulated by specific signaling pathways, such as the JAK-STAT pathway mentioned above. The Wnt/β-catenin signaling pathway is also heavily implicated, for example, through regulating cardiomyocyte differentiation of mouse ES cells, while the induction of ERK1/2 signaling triggers the differentiation of mouse ES cells towards the primitive endoderm lineage (39, 170). A schematic showing the proposed stem cell states is given in Figure 3.

Table 2. Examples of the disease models in which iPS cells have been used as a potential therapy for treatment

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cell of Origin</th>
<th>Factors</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell anemia</td>
<td>Tail-tip fibroblasts</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>86</td>
</tr>
<tr>
<td>Parkinson’s Disease</td>
<td>Patient-derived fibroblasts</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>226</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Patient-derived fibroblasts</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td>203</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>Patient-derived fibroblasts</td>
<td>Oct4, Sox2, Nanog, Lin28</td>
<td>59</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>Mouse embryonic fibroblasts</td>
<td>Oct4, Sox2, Klf4</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Patient-derived fibroblasts</td>
<td>Oct4, Sox2, Klf4, c-Myc</td>
<td></td>
</tr>
</tbody>
</table>
Signaling pathways therefore play a critical role in the induction of pluripotency during somatic cell reprogramming. However, unfortunately, rather little is known about the signaling pathways regulated by the pluripotency factors Oct4, Sox2, Klf4, and c-Myc, although recent work in mouse ES cells has identified key pathways that are regulated by these factors to maintain pluripotency (152). For example, work in ES cells has identified that two key signaling pathways are integrated into the Oct4, Sox2, and Nanog circuitries through Smad1 and STAT3 (40). Liu et al. (152) have also demonstrated that these pluripotency factors regulated a crucial developmental signaling network, which is comprised of 16 signaling pathways, including 9 with previously unknown roles in the maintenance of ES cell pluripotency. In addition, further work by Huang et al. (92) has identified a core signaling network regulated by these factors to induce pluripotency in reprogrammed somatic cells. This study identified eight pathways as essential pathways in iPSC and ES cells, including transforming growth factor (TGF)-β, Hedgehog, Wnt, p53, and JAK-STAT, and it is thought that the key pluripotency factors synergistically regulate these pathways in a balanced way to induce pluripotency (92). In addition, several other developmental signaling pathways, including mitogen-activated protein kinase (MAPK), vascular epidermal growth factor (VEGF), Notch, ErbB, and mTOR were identified as pathways supporting the core network (92).

We therefore have an emerging picture of pluripotency regulating factors which impinge on a broad range of signaling networks (FIG. 4). In the next section of this review, we will examine some of the key pathways identified as being important for iPSC cell induction. We will also investigate the established role of these pathways in maintaining ES cell pluripotency and their role in somatic stem cells. Although the core pluripotency network is thought to differ in adult stem cells, these key pathways have established roles in maintaining the somatic stem cells and in regulating tissue homeostasis; therefore, we will also examine the potential of these signaling pathways in tissue regeneration.

A. TGF-β

The TGF-β superfamily plays a major role in the biology of mammalian development and regulates many cellular processes including cell fate, proliferation, senescence, apoptosis, and tissue repair. TGF-β and associated members of the family are mainly pleiotropic growth factors and are comprised of two main groups, the bone morphogenetic proteins (BMPs) and the growth differentiation factors (GDFs), which includes TGF-β, activin, and nodal and can be further divided according to sequence homologies and functional similarities. The canonical signaling cascade of TGF-β growth factors involves the ligands of the TGF-β
superfamily binding to cell surface receptors that activate the Smad proteins in the cytoplasm, which leads to their nuclear translocation and transcriptional activation of target genes. This pathway is also regulated by other key signaling pathways such as Wnt and Hedgehog, and also other tyrosine kinase-linked growth receptor signaling pathways.

TGF-β signaling has been shown to be important in the maintenance of self-renewal and pluripotency of both mouse and human ES cells, although the role of these signaling molecules does appear to differ between the two species (258). In mouse ES cells, BMP4 maintains self-renewal through inhibition of the MAPK/ERK pathway by LIF and the expression of Id protein via the Smad pathway (198, 279). Furthermore, BMP4 promotes mouse ES cell proliferation via an increase in Wnt expression by Smad activation (137). In contrast, in human ES cells, BMP4 promotes stem cell differentiation through the downregulation of Nanog and Oct4, and activated Smad is only found at low levels (274). Long-term maintenance of human ES cell pluripotency therefore requires the downregulation of BMP activity by Noggin and basic fibroblast growth factor (bFGF) (6, 275). Other TGF-β family members are also thought to play an important role in the maintenance of ES cells. Phosphorylation and nuclear localization of Smad2 induced by TGF-β, activin, or nodal signaling was observed in undifferentiated human ES cells and was decreased upon early differentiation (103). The inhibition of these pathways initiated differentiation and resulted in the decreased expression of stem cell markers (103, 250). In addition, Xiao et al. (272) have demonstrated that activin is able to support long-term feeder-free culture and maintenance of pluripotency in human ES cells by inducing the expression of Oct-4 and Nanog, and suppressing BMP. Nodal expression also plays a role in the maintenance of human ES cell pluripotency through the inhibition of neuroectodermal differentiation, a default differentiation pathway of ES cells (251). In addition, activin and nodal signaling has been shown to promote mouse ES cell self-renewal in serum-free conditions (183). It is therefore clear that TGF-β signaling plays an important role in the maintenance of self-renewal and pluripotency, although the exact mechanism of action for this family of growth factors appears to differ between family members and species.

BMPs are also potent inhibitors of neural differentiation in vertebrate embryos (267), and it is thought that BMP regulation of ES cell pluripotency may be primarily through the

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**FIGURE 4.** Key developmental signaling pathways regulated by the core stem cell factors. These pathways have been shown to have a role in both ES cells and iPS cells and also in tumorigenesis (+). These pathways have also been shown to be essential in somatic stem cell maintenance and vertebrate limb regeneration. Examples of the identified role of these various pathways in key cellular systems are shown in the table.
inhibition of differentiation rather than as a direct stimulation of self-renewal (279). The knockdown of Smad1 and Smad4 expression in ES cells did not impair the self-renewal ability of these cells but led to a change in the expression pattern of germ layer markers during differentiation (66). Furthermore, Smad4−/− ES cells show no defect in their self-renewal ability (225). It has been suggested that BMP/Smad signaling interacts with the core self-renewal network in ES cells and modulates the balance between self-renewal and differentiation by regulating development-associated target genes. Consistent with this hypothesis, several Smad target genes overlap with genes bound by the key pluripotency factors, for example, Smad4-regulated genes have a substantial overlap with those of Sox2, NR0B1/Dax1, and Klf4 (66). In addition, a recent study has demonstrated that several Smad targets were mapped to Nanog, Oct4, and TCF3-bound genes (43).

TGF-β signaling also participates in the cell fate decisions of ES cells, and these factors can direct the fate of ES cells towards multiple cell lineages of the three embryonic layers, including neural, hematopoietic, cardiomyogenic, and hepatic (121). Specifically hematopoietic cell differentiation is dependent on BMP4 in mouse ES cells (190). BMP4 regulates mesodermal cell commitment to the hematopoietic lineage and specifies blood lineages at the later stages of differentiation (139, 190, 210). Furthermore, a recent study by Lengerke et al. (138) demonstrated that the role of BMP4 in hematopoietic differentiation is conserved in human iPSCs, and stimulation of BMP4 directs iPSC cell differentiation toward blood lineages.

TGF-β signaling also has a role during endothelial cell proliferation and vascular development in mouse ES cells (124, 180, 235, 259). BMP4 and activin are thought to induce mesodermal differentiation into the cardiac lineage and induce human ES cells to differentiate into cardiomyocytes (132). In addition, BMP2-induced mesodermal and cardiac specification results in full cardiogenic differentiation, leading to an enrichment of cardiomyocytes within embryoid bodies (20). This ability of the TGF-β family members to commit mouse ES cells toward a mesodermal fate is due to Smad-mediated regulation of the Oct4 promoter, further implicating a role for Smad signaling in the regulation of the core self-renewal network in ES cells (284). This role for TGF-β family members in cardiomyocyte differentiation is of potential interest for the treatment of cardiac disease, particularly as Narazaki et al. (172) have successfully differentiated mouse iPS cells into cardiovascular progenitor cells, which subsequently generated arterial, venous, and lymphatic endothelial cells as well as self-beating cardiomyocytes. It may therefore be possible to potentially manipulate the TGF-β pathway to increase the efficiency of cardiovascular cell differentiation from patient-specific iPS cells. In addition, the TGF-β pathway could potentially be used in the clinical treatment of other diseases by exploiting its ability to direct the cell fate of ES cells and potentially iPS cells. For example, recent progress in obtaining endoderm cells by treating differentiating ES cells with activin and BMP4 (78, 127) could potentially lead the way for the development of iPS cell use to obtain endoderm derivative cells such as pancreatic and hepatic cells for use in the treatment of type I diabetes and liver diseases, respectively.

### B. TGF-β and In Vivo Regeneration

In addition to its role in ES cells, the TGF-β signaling family also has a functional role in the maintenance of somatic stem cells and also tissue regeneration (258). This role of the TGF-β pathway is of particular significance as the potential use of iPS cells in regenerative therapy requires a clearer understanding of the regulatory signaling networks maintaining somatic stem cells and driving tissue regeneration. BMP signaling plays a vital role in the regeneration of the intestinal epithelium. The BMP pathway maintains intestinal stem cell quiescence and regulates terminal differentiation by negatively regulating the Wnt signaling pathway through PTEN activation. The conditional deletion of Bmpr1a results in the expansion of the stem and progenitor cell populations leading to hyperproliferativecrypts, and the inhibition of BMP signaling on the villus through overexpression of Noggin results in ectopic crypt formation (88). BMP antagonism of the Wnt pathway also maintains the hair follicle stem cells (74, 122, 144), the mesenchymal stem cells (104, 153, 174), and neural stem cells (64). In all these systems, BMP signaling molecules antagonize Wnt signaling to maintain quiescent stem cells.

In addition to its role in somatic stem cell maintenance, BMP signaling also has a functional role during limb regeneration. BMP is required for *Xenopus* tail and limb regeneration (17, 80) and also mammalian digit tip regeneration (83). During *Xenopus* tail regeneration, BMP signaling is required to induce cellular proliferation in the notochord and the spinal cord (17). BMP also has the ability to rescue regeneration of the entire tail when overexpressed, suggesting that BMP regulation of these structures may be the driving force behind tail tissue regeneration (17). BMP is also essential for bone matrix deposition by regulating the proliferation and/or differentiation of scleroblasts during the outgrowth phase of zebrafish fin regeneration (200). In addition to appendage regeneration, BMP signaling has also been shown to be crucial in the regulation of early events during liver regeneration in zebrafish (108).

### C. WNT

Similar to TGF-β signaling, the Wnt pathway has an important role in tissue development and also regulates cellular mechanisms such as adhesion, morphology, proliferation,
The Wnt signaling pathway is coupled directly with the core transcriptional network of pluripotency. Wnt signaling is activated in ES cells and is downregulated during differentiation (214). Sato et al. (214) demonstrated that the activation of the canonical Wnt pathway is sufficient to maintain self-renewal of both human and mouse embryonic stem cells and is independent from the LIF/STAT path- way. During this study, they demonstrated that the activation of the Wnt signaling pathway maintained pluripotency and also sustained the expression of pluripotency factors including Oct4, Nanog, and Rex1. Further studies also demonstrated that the conditioned medium from mouse fibroblasts expressing Wnt maintained the pluripotency of mouse ES cells (182). In addition, a study overexpressing an activated mutant of β-catenin confirmed the role of Wnt in maintaining the expression levels of Oct4, Nanog, and Rex1 and the long-term proliferation of ES cells (238). This study also demonstrated that the β-catenin upregulation of Nanog was in an Oct4-dependent manner and that β-catenin physically associates with Oct4 (238).

In addition to maintaining pluripotency, Wnt signaling regulates differentiation in ES cells. Wnt signaling has been found to specifically inhibit neural differentiation (12, 82), and several other studies have implicated that Wnt signaling may be sufficient to inhibit differentiation altogether (214). ES cells with a mutated form of Apc were found to exhibit differentiation defects both in vitro and in teratomas, and this inhibitory effect was shown to be dose-dependent with the severity of the Apc mutation (115). ES cells with highly elevated β-catenin levels also have a compromised ability to differentiate (54), although even mutant ES cells with the highest levels of β-catenin activity were capable of some differentiation, which is consistent with previous reports that the activation of Wnt signaling alone is not sufficient to support long-term ES cell self-renewal (182, 280).

The downstream mediators of β-catenin activation are the TCF/LEF family of transcription factors. In ES cells, Tcf3 is the most abundantly expressed member of this transcription factor family, and Tcf3-null ES cells have an increased resistance to differentiation (195). Tcf3-null ES cells showed upregulation of various Oct4- and Nanog-regulated genes and was also shown to cooccupy the promoters of several Oct4 and Nanog targets (43). TCF3 is thought to function to oppose the effects of the core pluripotency machinery and limit the expression of key regulators. Activation of Wnt converts TCF3 into an activator, elevating the expression of these same targets and suppressing differentiation (43). TCF3 may also suppress the expression of Oct4 (240) and Nanog (195), although its ability to activate these targets is unclear.

Recent studies have also established a role for Wnt signaling in the generation of iPS cells. Soluble Wnt3a promotes the derivation of iPS cells in the absence of c-Myc (163). Also, the simultaneous inhibition of MAPK pathway and glyco- gen synthase kinase-3 (GSK3) promotes iPS cell formation from brain-derived NSCs, using only Oct4 and Klf4 (223). These pathways may facilitate iPS cell generation by activating endogenous reprogramming factors or pluripotency-associated genes, and further investigations are required to understand how Wnt may regulate somatic cell reprogram- ming.

### D. Wnt and In Vivo Regeneration

The role of Wnt in ES cells and iPS cell reprogramming suggests that manipulation of this pathway could have the potential to be beneficial for regenerative therapies. Wnt signaling has a vital role in regulating cell growth and differentiation and is essential in the maintenance and self-renewal of various somatic stem cells and is therefore vital to the regeneration of these tissues. For example, the constant proliferative state of the stem cells in the intestinal crypt is under the control of the Wnt pathway, and loss of Wnt signaling results in loss of the stem cell compartment (125, 128). Knockout mice deficient in Tcf4 lack crypt structures, and the entire intestinal epithelium is composed of nondividing villus cells (125). Similar to the situation in ES cell maintenance, Oct4 plays an important role in intestinal crypt maintenance as ectopic, intestinal expression of Oct4 results in an increase in β-catenin expression, dyspla- sia, and an expansion of crypt progenitor cells (91). This phenotype is similar to that seen following conditional loss of Apc (213). Critically, Wnt signaling has also been shown to play a direct role in the regenerative response after injury. Thus treatment with the positive Wnt modulator R-Spondin1 was able to restore the damaged mucosa in both experimental colitis and radiation-induced oral mucositis (287, 288). This role in the regenerative response has recently shown to be Wnt dependent, being mediated down- stream of c-Myc by focal adhesion kinase (11).

The requirement for Wnt signaling is, however, not con- strained to the gastrointestinal tract, with other epithelial organs such as the lung (286) showing Wnt-dependent control of the balance between progenitor expansion and epi-
the regenerative response is reduced and in nonregenerative
ApcMin/erated liver regeneration after a partial hepatectomy (77).
Stabilization of β-catenin also drives hair follicle stem cells to proliferate (157), and overexpression of Wnt ligand in the epidermis is able to induce hair follicle regeneration after wounding (100).

Wnt signaling has also been suggested to play a role in mammalian liver regeneration as patients with mutations in the APC gene have an increased risk of developing hepatoblastoma, indicating that Wnt affects the hepatic progenitor cells (75, 130, 181). Work in zebrafish has also demonstrated that an increase in Wnt activity significantly accelerated liver regeneration after a partial hepatectomy (77). Furthermore, liver regeneration is significantly enhanced in ApcMin/+ mice, and a conditional knockout model of β-catenin in the mouse liver has demonstrated decreased hepatocyte proliferation and suboptimal regeneration (241). In addition, Wnt signaling is upregulated in adult hepatic progenitor cells during regeneration (101, 278). Wnt signaling can also promote the renewal of the hematopoietic stem cells, and studies have shown that the overexpression of activated β-catenin results in stem cell survival and proliferation (205). In addition, during the regenerative process following injury, hematopoietic cells reactivate growth signals and show an increased activation in Wnt signaling (46).

Several studies have also demonstrated the essential role of Wnt signaling in the regeneration of mammalian muscle and bone (219, 290). For example, the Wnt pathway plays a vital role in bone homeostasis and has a role in the normal embryonic development of the vertebrate limb (95). Wnt signaling is also activated during fracture repair and enhances bone formation (222). Furthermore, gain-of-function mutations in the Wnt coreceptor LRPS in humans gives rise to high bone mass (13). A recent study has also demonstrated that modulation of Wnt levels through Dkk1 inhibition was sufficient to repair the bone lesions in multiple phases of fin regeneration and is sufficient to enhance regenerative outgrowth; also, β-catenin-independent Wnt signaling functions as an antagonist of fin regeneration, allowing a negative feedback loop to control fin regrowth (232). Similarly, a crucial role for Wnt has been demonstrated in Xenopus tail regeneration with the molecular mechanism being similar to that of the zebrafish fin (149, 234). The only mammalian organ capable of complete regeneration, deer antlers, also requires β-catenin expression to maintain the survival of antler progenitor cells (169).

Work in zebrafish has also demonstrated a functional role for Wnt/β-catenin in heart regeneration, which has been confirmed by studies in mice. In vivo studies using β-catenin conditional heart specific null mice has demonstrated a role for the canonical Wnt signaling pathway in the differentiation of cardiomyocytes (3, 131). The Wnt11 ligand is required for cardiac differentiation and heart formation in both chick and Xenopus embryos (60, 189). This function of Wnt11 in heart development has also been shown to be conserved in higher vertebrates as exogenous Wnt11 promotes the differentiation of murine ES cells into cardiomyocytes (245).

From the studies discussed above, it is clear that Wnt/β-catenin signaling plays a central role in many regenerative processes, suggesting that Wnt/β-catenin signaling may be a conserved feature of regeneration. The manipulation of this pathway should therefore be key in both manipulating somatic stem cells in situ and also in the development of iPSC cells for regenerative therapy.

E. Hedgehog

In vertebrates the Hedgehog (Hh) gene family encodes three highly conserved, homologous signaling proteins: Sonic (Shh), Indian (Ihh), and Desert (Dhh). These signaling molecules bind to the membrane receptor Patched (Ptc), which in the absence of Hh inhibits the activity of another transmembrane protein, Smoothened (Smo) (99). The downstream effectors of this pathway are the Gli family of zinc-finger transcription factors, and the alteration of the balance between activator and repressor forms of these proteins results in regulation of Hh target genes.

Hh signaling is vital for many developmental processes, and Shh has been shown to be involved in the determination of cell fate and embryonic patterning during early vertebrate development.

F. Hedgehog and In Vivo Regeneration

Hh signaling is important for the maintenance of stem or progenitor cells in many adult tissues, including the epithe-
lia of many internal organs and the brain (16, 254). In the small intestine, Hh signaling is important for the correct overall crypt-villi structure of the epithelium, although it is not directly involved in the fate of the epithelial cells (159). Inhibition of Hh signaling in the intestinal epithelium results in reduced villus formation and mislocalization of subepithelial stromal cells. In addition, loss of Hh signaling has a secondary effect by enhancing proliferation through Tcf4 and β-catenin gene activity and the formation of ectopic precrypt structures on villus tips (159). In addition to tissue maintenance through Hh-mediated stem cell or progenitor cell self-renewal, Hh signaling also plays a critical role in the regenerative responses to tissue injury, for example, in the regeneration of the pulmonary epithelium (260), prostate epithelium (111), and exocrine pancreas (67). In addition, a recent study has demonstrated a role for Hh signaling during gastric regeneration, where this pathway is essential for the differentiation of gastric progenitor cells (110). Recent work has also suggested a role for the Hh pathway in hepatic remodeling as liver injury increases the activity of Hh signaling in progenitor cells (69, 187).

Hh signaling also functions to maintain adult hematopoietic stem cells (HSCs) in both Drosophila and vertebrates by regulating the HSC cell cycle and by balancing hematopoietic homeostasis and regeneration in vivo. Loss of Hh signaling in Drosophila results in the depletion of blood progenitor cells in the lymph gland due to premature differentiation (162) and the inhibition of hematopoiesis and vasculogenesis in early postimplantation mouse embryos (58). In adult mice, Ihh is expressed in differentiated epithelial cells of the small intestine, and conditional of Ihh from these cells results in a wound healing response, thought to be mediated through mesenchymal signaling (252). Ihh is also expressed in bone marrow stroma, while Shh expression is found in lymph node and spleen stroma. Ihh overexpression in stromal cells promotes hematopoietic regeneration after bone marrow transplantation (123), and Hh signal activation induces cycling and expansion of primitive hematopoietic cells (247).

The Hh pathway has also been extensively analyzed in neural stem and progenitor cells and has several roles in the specification, proliferation, and differentiation of neural precursors during embryogenesis. Hh signaling is also required for the maintenance of quiescent neural stem cells in the adult brain (2, 14, 188). Shh has been shown to be crucial in regulating stem cell niches and neural stem cell proliferation in the postnatal telecephalon (244), the adult hippocampus (133), and the SVZ (2) and plays a critical role in the survival and patterning of neural progenitor cells in the ventral spinal cord (151).

Hh signaling also has a role in vertebrate limb development and is vital to both bone formation and bone remodeling, which is primarily mediated through the regulation of both chondrocyte and osteoblast differentiation (50). This function of the Hh pathway, in addition to its role in wound healing, suggests a potential role for the Hh pathway in limb regeneration. Consistent with this, work in urodèles has demonstrated an increase in Shh expression after limb amputation (98, 246) and also in the regenerating limb buds of anurans (62). Functional evidence also suggests that Shh is important for anterior-posterior axis patterning in the regenerating limb (208, 209). Furthermore, activating Shh in Xenopus frogs improves limb regeneration and results in the formation of multiple cartilaginous structures (276). Shh is also required for dorsoventral patterning of the regenerating spinal cord and surrounding mesodermal tissues during Xenopus tail regeneration (216). While this function is similar to that seen in the regenerating limb, it is clear that the Shh signaling pathway has distinct roles in different regenerating organs. Zebrafish fin regeneration also requires Shh during the outgrowth phase, and studies have implicated a role for this pathway in the proliferation and/or differentiation of scleroblasts (200). Shh has also been shown to be important in axolotl tail regeneration, where it is required for dorsoventral patterning of the regenerating spinal cord and for the regeneration of surrounding mesodermal tissues (216).

From the studies described above, it is clear that the Hedgehog pathway is a fundamental regulator of somatic stem cells that maintain tissue integrity in mature organisms. The importance of hedgehog signaling in regeneration has been emphasized by recent work developing hedgehog-related therapies for restoring tissue function where the normal capacity to regenerate has been compromised (256).

G. JAK-STAT

The JAK-STAT pathway plays an essential role in mediating the biological response to growth factors and cytokines, and characterization of IFN signaling resulted in the initial identification of this pathway. Currently, four Janus kinase (JAK) family members have been identified, and seven signal transducers and activators of transcription (STATs) are known. The binding of ligands to the dimeric cytokine receptor results in the activation of two receptor-associated JAKs and subsequent phosphorylation of the ligand-bound receptor. The phosphorylation of the receptor tyrosine residues promotes the recruitment of a STAT family member, which becomes phosphorylated and forms a homo- or heterodimer, which translocates into the nucleus and activates target genes.

The JAK-STAT pathway plays a vital role in maintaining the pluripotent state of mouse ES cells. As described above, in vitro culture of mouse ES cells requires LIF to maintain pluripotency. The JAK-STAT pathway is a key downstream mediator of LIF, and the activation of the STAT3 pathway by LIF induces transcription of self-renewal genes (34, 177).
STAT3 is the primary STAT protein activated in mouse ES cells, and constitutively active STAT3 removes the need for LIF to promote self-renewal (177, 178). STAT3 has multiple roles in the regulation of ES cell pluripotency including gene activation, cell cycle regulation, and the inhibition of differentiation pathways. STAT3 has also been reported to function through the regulation of c-Myc (35), Klf4 (147), and Klf5 (25), although these target genes have not been shown to be completely sufficient to replace the effect of LIF. This ability of LIF-mediated activation of STAT3 to support the long-term self-renewal of mouse ES cells in vitro has been supported in vivo by the requirement for this pathway in embryonic diapause (175). In addition to STAT3 homodimers, STAT1 is able to heterodimerize with STAT3 in mouse ES cells, although it is thought that it is unlikely to be required for self-renewal as Stat1−/− cells remain LIF-dependent for undifferentiated growth (56). In human ES cells, STAT3 does not appear to be sufficient to maintain pluripotency (214), indicating once again that the signaling pathways responsible for maintaining pluripotency may be species-specific. Also, recent work by Griffiths et al. (79) has identified a STAT3-independent JAK pathway that is able to directly regulate pluripotency genes and maintain self-renewal through chromatin phosphorylation.

H. JAK-STAT and In Vivo Regeneration

In addition to maintaining pluripotency, the JAK-STAT pathway stimulates cell proliferation, differentiation, cell migration, and apoptosis and is vital in regulating various developmental processes (97).

Recent work has demonstrated a key role for JAK-STAT signaling in the homeostasis of the Drosophila midgut. The JAK-STAT pathway regulates ISC self-renewal and differentiation, and compromised JAK-STAT signaling causes ISC quiescence and loss, whereas signaling overactivation produces extra ISC-like and progenitor cells (150, 239). In addition to midgut ISCs, Stat92e is also specifically activated in the Drosophila hindgut stem cell population (239), indicating a similar role of JAK/STAT signaling in hindgut ISCs. Several recent studies have also suggested a role for Drosophila JAK-STAT signaling in mediating injury- or infection-induced ISC proliferation and regeneration (5, 31, 49, 105, 179). Enterocytes that are subjected to stress produce cytokines that activate the JAK-STAT pathway in the intestinal stem cells, which results in proliferation and regeneration (105). Furthermore, we have shown that conditional deletion of Stat3 in the mouse intestinal epithelium leads to loss of the stem cell compartment (unpublished data), while others have shown that in a mouse model of DSS-induced colitis, colon epithelial damage triggers an immune response and the expressed cytokines activate STAT3, which subsequently promotes cell proliferation and regeneration. In addition, inhibition of the STAT inhibitor SOCS3 in the mouse gut increased the proliferative response to DSS (207).

The canonical JAK-STAT pathway also regulates germ line stem cell self-renewal, maintenance, and differentiation in the Drosophila (29, 116, 248). The JAK-STAT pathway maintains germ line stem cell self-renewal by activating the expression of self-renewal genes, and conditional loss of JAK-STAT signaling results in germ line stem cells differentiating into spermatogonia without self-renewal (29). Furthermore, the conditional restoration of JAK-STAT signaling in differentiated spermatogonia that are undergoing transit-amplifying divisions are able to dedifferentiate to become functional germ line stem cells that are able to repopulate the niche (29).

STAT3 influences cell survival, metabolism, growth, differentiation, and migration in multiple organs, and recent work has indicated a vital role for STAT3 in liver regeneration (145). In the normal liver, the interleukin (IL)-6/STAT3 pathway is thought to play a central role in regeneration as disruption of this pathway in IL-6-deficient mice results in impaired liver regeneration after 70% partial hepatectomy (PH) (48). STAT3 signaling also has a vital role in wound healing, and in vivo studies have revealed that Stat3 expression in keratinocytes contributes to the regeneration of the epidermis and hair cycle process (212). This potential role of the STAT3 pathway in wound healing has also been observed in the injured spinal cord. After injury, STAT3 activation (phosphorylation and nuclear localization) was observed in reactive astrocytes surrounding the lesion, implicating a role for this pathway in tissue repair and functional recovery (185).

I. Notch

The Notch pathway is an evolutionary conserved pathway that regulates cell fate determination during development. This pathway is essential in the control of embryonic organogenesis and is also vital for postnatal tissue repair (10). The Notch pathway consists of a single transmembrane receptor, which undergoes proteolytic cleavage upon binding by members of the Jagged and Delta ligand families. Cleavage involves the activity of the γ-secretase protease complex and releases a Notch intracellular domain (NICD) that translocates to the nucleus. In the nucleus, NICD heterodimerizes with CSL transcription factors and activates Notch target genes (30).

Notch signaling is required for the differentiation of ESCs; however, the precise function of endogenous Notch signaling in mammalian ESCs is still unclear, although several components of the Notch signaling pathway are highly expressed in both mESCs and hESCs (206, 215). Notch signaling is thought to be relatively inactive in undifferentiated hESCs, and not necessary for their proliferation, although many hESC-derived differentiated cells require Notch (179). Neural specification of ESCs is influenced by Notch signaling, and Notch1 activation promotes neural lineages...
and suppresses ESC differentiation into mesodermal cell fates (173, 218). Conversely, the inhibition of Notch reduces neural lineage differentiation and increases nonneural ESC fate. For example, ESC differentiation into cardiomyocytes required Notch1 inhibition (173).

J. Notch and In Vivo Regeneration

In addition to a role in embryonic stem cells, Notch signaling is required for the maintenance and repair of various organ systems. The Notch pathway is required for self-renewal and cell-fate commitment of tissue stem cells and has been shown to play a vital role in the regenerative response to injury in several adult tissues including the kidney, liver, bone marrow, skin, muscle, intestine, heart, trachea, and pancreas.

In the small intestine, the Notch pathway plays a central function in the cell fate decisions of intestinal stem cells, and similar to the Wnt pathway, the Notch pathway is essential to maintain the crypt compartment in its undifferentiated, proliferative state. Inhibition of various components of the Notch pathway in the intestine results in the rapid and complete conversion of all epithelial cells into secretory goblet cells (167, 270), an effect which is also seen in intestine, liver, bone marrow, skin, muscle, intestine, heart, trachea, and pancreas.

The ability of Notch to expand the stem cell population in response to injury has also been shown in several other tissues including the brain (242), and various studies have confirmed that the capacity of Notch to enhance tissue regeneration is due to its ability to activate progenitor cell populations, in addition to regulating the differentiation status of cells and enhancing proliferation. Therefore, the activation of Notch signaling may provide a promising target for regenerative therapy in adult tissue in response to injury.

Notch signaling is also vital for the functional recovery of vertebrate appendages. The inhibition of Notch prevents anuran tail regeneration, while the overexpression of NICD stimulates the regeneration of a tail that contains notochord and spinal cord but little or no muscle (18). Notch also has a role in mammalian skeletal muscle regeneration, and Notch signaling is induced in activated muscle progenitor cells, satellite cells, 2 days after injury both in culture and in vivo (45). Expression of a constitutively active Notch receptor results in the inhibition of differentiation markers and increased proliferation of progenitor cells in culture (45).

Importantly, the inhibition of Notch signaling in vivo impairs muscle regeneration (44). The induction of Notch signaling in activated satellite cells appears to be triggered by the upregulation of the Delta ligand in satellite cells themselves and in muscle fibres adjacent to the damage (44). In addition, the upregulation of Delta and the activation of Notch are diminished in old mice, whose satellite cells proliferate less in response to injury, resulting in reduced efficacy of regeneration. Forced activation of Notch signaling at the site of injury in old mice is sufficient to improve muscle regeneration, rendering it similar to that of young mice (44).

K. FGF/ERK MAPK Pathway

The MAPks are a family of serine/threonine kinases that have a functional role in connecting the activation of cell-surface receptors to various cellular functions such as proliferation, differentiation, migration, and apoptosis (38, 194). MAPks are a part of a three-tiered kinase module that consists of a MAPK, an upstream MEK, and a MEKK that is coupled to cell surface receptors via adaptor proteins. The ERK MAPks can be activated by a wide variety of extracellular ligands, including growth factors, serum, cytokines, and stress signals. One important upstream activator of the ERK MAPK pathway is the fibroblast growth factor (FGF) family of signaling molecules. FGFs are a large family of signaling molecules with various functions in development and adult physiology, and the ERK pathway mediates the effects of this growth factor.

In human ES cells, it has been well established that exogenous FGF-2 is required to sustain self-renewal, and it is widely used to culture human ES cells and iPS cells (6, 52, 273). Furthermore, in human ES cells, elevated levels of exogenous FGF-2 alone are sufficient to maintain undifferentiated hESC in the absence of fibroblast feeders or fibroblast-conditioned media (141, 275). Inhibition of FGF signaling in hESC results in rapid differentiation (57), an effect that is similarly seen in mouse ES cells (268). In human ES cells, exogenous FGF-2 activates the ERK MAPK pathway, which is thought to be necessary for the maintenance of
pluripotency, although the mechanism of action is still unclear (57, 143).

In contrast to most cell lines and human ES cells, undifferentiated mouse ES cells do not require the ERK pathway for proliferation and self-renewal (33), and remarkably, ERK1/2 signaling actually inhibits the self-renewal of these ES cells and triggers differentiation towards the primitive endoderm lineage (39). Furthermore, reducing ERK activity has been shown to enhance the efficiency of ES cell derivation by promoting the retention of Oct4-positive epiblast during the outgrowth phase (32). Also, the differentiation of established ES cells and their requirement for LIF can be diminished by the addition of synthetic inhibitors of ERK signaling (34). The mechanism behind the ability of the ERK pathway to promote differentiation is unclear, although recent studies have suggested that ERK1/2 activation may act as a stimulus for ground-state ES cells, which are intrinsically self-maintaining, to exit the self-renewal program and enter a state that is responsive to inductive stimuli (129). As mentioned already, the direct consequence of this is that blocking the ERK inductive differentiation pathway can maintain the ground state of the epiblast (280). As perhaps a direct consequence of the above, this inhibition of Erk1/2 MAPK pathway has been shown to significantly improve the generation efficiency of iPS cells (92, 223).

L. FGF/ERK and In Vivo Regeneration

As described above, the ERK MAPK family is activated by several growth factors, one of which is FGF. The FGF pathway plays a central role in development and tissue regeneration, and FGF signaling has been implicated in the regulation of cell proliferation and specification in the majority of regenerating systems. Components of the FGF pathway are expressed in various somatic stem cell compartments, and FGFs appear to be universal regulators of regeneration.

In the mouse small intestine, FGFs have been shown to have an active role in murine colon development and have been shown to promote the proliferation and survival of intestinal cells (211). The FGF receptor FGFR3 has been shown to be strictly expressed in the intestinal progenitor cell compartment, where it has a role in regulating the expansion of intestinal crypt cells (9). Also, in irradiated mice, treatment with FGF7 increases crypt survival (114), and FGF1 and FGF2 have a protective effect after intestinal injury (51, 71). In humans, various FGF receptors have been shown to be expressed in the gastrointestinal tract, and the altered expression of FGFR3-IIIb in intestinal epithelial cells is associated with differentiation (109).

In addition to tissue maintenance, FGF signaling is important in limb regeneration after injury. FGFs have several essential roles during epimorphic regeneration of anuran tails, urodele limbs, and fish fins and regulate the various stages including wound epidermis formation, blastema formation, and proliferation and also positional memory (231). The FGF pathway is vital during Xenopus limb regeneration, and the treatment of a nonregenerative stage Xenopus limb stump after amputation with FGF8 results in partial regeneration (281). Furthermore, treatment with FGF10 results in significant regeneration of the limb due to the activation of expression of several genes that are expressed in regenerating limbs such as Shh and Fgf10 (281). Also, the treatment of the amputation surface of a nonregenerative chick limb bud with FGF2 or FGF4 induces a regenerative response (126, 243). FGF signaling is also active and important in all three stages of zebrafish fin regeneration, similar to the Wnt pathway (231), and also for the later phases of heart regeneration (140). FGF signaling is also implicated in the regulation of cell proliferation and differentiation in regenerating skeletal muscle and mammalian liver (63, 233), although clarification of its in vivo role is still required.

VI. REGENERATIVE PATHWAYS

The signaling networks that play a vital role in stem cell maintenance of ES cells and somatic stem cells are essential for progenitor cell formation and for the differentiation of cells leading to tissue homeostasis. By looking at the pathways that regulate pluripotency, self-renewal, and stem cell-mediated regenerative processes, it should enable us to progress the dual tracks of in situ stem cell manipulation and the development of iPS cells for regenerative therapy. A key goal of these studies must be the identification of small molecules that can be used to precisely manipulate these regenerative processes.

Although we have briefly discussed some of the potential pathways involved in pluripotency maintenance and also their potential role in tissue regeneration, it is important to remember that these pathways do not function in isolation from each other or from other signaling pathways (e.g., see FIG. 4). For example, the effects of Wnt signaling on stem cells are modulated through association with Notch, Sonic Hedgehog, and TGF-β (41). In addition, several other key pathways regularly appear to be essential in the various regenerative processes discussed above that have not been discussed in this review. For example, cell cycle control pathways have a vital role in tissue regeneration, and several studies have demonstrated that checkpoint proteins such as p21 and Rb also appear to be important in tissue regeneration in mice (19, 89, 217, 230, 266).

In addition, it is important to note that stem cells also depend on the extrinsic signaling pathways of the stem cell niche. This stem cell microenvironment plays a vital role in deciding the fate of these cells. The niche provides supportive growth factors and cytokines for the stem cell and con-
tributes to the maintenance of the undifferentiated stem cell state. For example, regulation of the intestinal stem cell involves constant cross-talk between the epithelial cells and the underlying mesenchymal cells in the intestinal stem cell niche. This cross-talk is mediated by some of the key pathways mentioned above, in addition to pathways such as the phosphatidylinositol 3-kinase pathway. This additional level of control emphasizes yet further the complex goal of precisely manipulating the endogenous stem cell population.

VII. REGENERATION OR TRANSFORMATION?

The discussion above has focused on the positive roles of six key pathways in pluripotency and regeneration. However, these self-renewal pathways are also key pathways that often become deregulated in tumorigenesis. For example, activating mutations in the Wnt/β-catenin pathway have been shown to initiate tumorigenesis in a variety of human epithelial carcinomas, possibly by expanding the stem cell population as has been suggested in the small intestine (204). Similarly, activation of Wnt signaling has been shown in non-small-cell lung cancer (249), and bronchioalveolar stem cells have been shown to multiply in response to Wnt activation (286). Therefore, although the manipulation of these pathways may be vital to the development of iPS cell technology and regenerative therapy, there is clear potential for unwanted side effects.

Several studies have made the experimental observation that, in some circumstances, it is possible to enrich for cancer forming cells within a bulk population of tumor cells, which has led to the concept that cancers may be sustained through a small population of “cancer stem cells.” This model suggests that tumors are generated and maintained by a subset of undifferentiated cells, the cancer stem cells, which are similar to adult tissue-specific stem cells and are able to self-renew and differentiate into the bulk tumor population (see FIG. 5). Although the origin and indeed the existence of the cancer stem cell is still under debate (53, 76, 81, 202), it is thought that the cancer stem cell could possibly arise from adult stem or progenitor cells that, due to their higher capacity to proliferate, are more prone to accumulate mutations during their lifespan. These observations suggest that deregulation of the pathways such as the ones discussed above, that control “stemness” may potentially promote cancer, either by increasing the number of potential initiators of neoplasia, or by expanding an established cancer stem cell.

The cancer stem cell hypothesis has gained strength through the apparent identification of initiating cell populations in several tumor types, including blood (134), breast (4), pan-

![FIGURE 5. The cancer stem cell model. The cancer stem may occur as a result of acquired mutations in a somatic stem cell. The cancer stem cells retain the ability to self-renew and through multiple asymmetric cell divisions are able to produce daughter cells that may form the bulk tumor population.](http://physrev.physiology.org/attachment.php?attachment_id=4559)
creas (135, 142), as well as colon and brain (224). This field does, however, remain contentious, with some arguing that the observation of such initiating cells occurs as an artifact of the experimental protocols used. Despite this uncertainty, it remains clear that early progenitor or stem cells in a range of tumor types can undergo oncogenic transformation, and it therefore seems highly likely that the modulation of the pluripotency pathways of these cell populations will directly alter tumor predisposition.

Several of the pathways discussed in this review have already been shown to be deregulated in cancer stem cells, and thus manipulating these pathways may be a double-edged sword. Some examples of these involvements are given in Table 3. For example, the Hedgehog pathway has been shown to be active in the cancer stem cells of several myeloma (193) and brain tumors (15, 42, 197, 201). Direct modulation of tumor outcome has now been shown for the myeloma (193) and brain tumors (15, 42, 197, 201). Direct modulation of tumor has been shown to be active in the cancer stem cells of several myeloma (193) and brain tumors (15, 42, 197, 201). Direct modulation of tumor outcome has now been shown for the Wnt pathway, which has been suggested to sustain stemness of CSCs within cutaneous squamous cell carcinomas, such that deletion of β-catenin in established tumors resulted in rapid loss of the CSC compartment and tumor regression (161). Conversely, increased BMP4 signaling has been shown to block glioblastoma formation by inhibiting cell proliferation and promoting CSC differentiation (196). Critically, in this example, the in vivo delivery of BMP4 was able to reduce tumor growth and the associated mortality in mice inoculated with human glioblastoma cells (196). In a similar way, epigenetic silencing of the BMP type IB receptor is thought to contribute to the pathogenesis of glioblas-

Table 3. Examples of the mutations that occur in the key self-renewal pathways and their incidence in various cancers

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Cancer/Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β</td>
<td>↓ SMAD4 in 53% of pancreatic carcinomas</td>
</tr>
<tr>
<td></td>
<td>↑ BMP2 in 98% of lung carcinomas</td>
</tr>
<tr>
<td>Wnt</td>
<td>↑ β-Catenin in 48% of small intestinal carcinomas</td>
</tr>
<tr>
<td></td>
<td>↓ APC in 90% of colon cancers</td>
</tr>
<tr>
<td></td>
<td>↓ APC in 76% of gastric adenomas</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>↓ Ptcn in 67% of basal cell carcinomas</td>
</tr>
<tr>
<td></td>
<td>↓ Smo in 10% of basal cell carcinomas</td>
</tr>
<tr>
<td></td>
<td>↓ Ptcn in 10-20% of sporadic medulloblastomas</td>
</tr>
<tr>
<td>JAK-STAT</td>
<td>↑ STAT3 in 50% lung</td>
</tr>
<tr>
<td></td>
<td>95% head and neck</td>
</tr>
<tr>
<td>Notch</td>
<td>↑ Notch in 55-60% of T-cell acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>MAPK-ERK</td>
<td>↑ RAS in 45% of colon and 90% of pancreatic cancers</td>
</tr>
<tr>
<td></td>
<td>↑ RAF in 60% of melanoma</td>
</tr>
</tbody>
</table>

[Adapted from Dreesen and Brivanlou (55).]

The examples cited above are but a few of the many examples implicating aberrant control of stem cell signaling pathways in the initiation and progression of cancer, either through the expansion of normal or preneoplastic stem cells, or by the expansion of proposed cancer stem cell populations. The cancer stem cell hypothesis remains controversial, and while the discussion of both sides of the argument is beyond the scope of this review, the potential existence of a tumor initiating cell population must remain a key consideration for any therapies based on either modifying stem cell signaling pathways and the stem cell niche directly in vivo or the introduction of iPS cells.

VIII. SUMMARY

Manipulation of the stem cell compartment remains an attractive target for the development of novel regenerative therapies, either by directly manipulating existing stem cells in vivo, or by supplying exogenously developed iPS cells. This problem might usefully be broken down into two fundamental questions: 1) Have we identified the key molecules and pathways that regulate stemness? 2) Can the manipulation of these pathways lead to controlled tissue regeneration? We have already made substantial progress towards addressing the first question, with a good understanding of the factors that control pluripotency, together with an understanding of the pathways that they compose. The main difficulty in addressing the second question is simply the ubiquitous nature of the programming machinery; as we have tried to summarize in this review, it spans and interacts with many, if not all, of the key transcriptional pathways that regulate cellular life. For example, many laboratories have demonstrated a role for the Wnt pathway in both pluripotency and repair, but unfortunately, the Wnt pathway is so broad in its downstream influence that we will need very complete and precise knowledge of the checks and balances that influence Wnt-mediated regeneration before we can think of using this pathway therapeutically. This problem is further compounded by the extra level of control exerted epigenetically, and also by the clear influence of the niche within which the stem cells sit.

Hence, the real difficulty may be in understanding and integrating these pathways in a single tissue type or setting. For example, complete deregulation of the Wnt pathway fails to lead to a simple enhancement of a stem cell or regenerative phenotype, but rather to a more sinister preneoplastic phenotype. More specifically, overexpression of the Wnt-related pluripotency gene Oct4 also leads to a preneoplastic phenotype rather than an enhanced regenerative phenotype. The conclusion that can be perhaps drawn is that simple deregulation of the stem cell program will default to an enhanced predisposition to neoplasia precisely.
because we have failed to understand the complete network that regulates entry and exit into the stem cell compartment.

Despite these clear difficulties, we are successfully beginning to understand these networks more fully, and we do now have clear examples of pathway-driven regeneration in mammalian tissues. Indeed, we absolutely know from other organisms that regeneration driven by these pathways is possible, and the challenge now must be to develop a systems understanding of the multiple processes governing regeneration so that we can ultimately utilize them therapeutically.

ACKNOWLEDGMENTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


STEM CELL COMPARTMENT, REGENERATION, AND PLURIPOTENCY PATHWAYS


